UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF PREVENTION, PESTICIDES AND TOXIC SUBSTANCES

TXR# 0054120

DATE: August 17, 2006

MEMORANDUM

SUBJECT: **BIFENTHRIN** - Review of Developmental Neurotoxicity Study in Rats (MRIDs 46750501 and 46750502)

PC Code: 128825
DP Barcode #: D327148

From: Robert J. Mitkus, PhD
Registration Action Branch I
Health Effects Division (7509P)

Thru: P.V. Shah, PhD, Branch Senior Scientist
Registration Action Branch I
Health Effects Division (7509P)

To: George Larocca
Insecticide-Rodenticide Branch
Registration Division (7505P)

ACTION REQUESTED: The Registration Division (RD) requested the Health Effects Division (HED) to perform a review of a developmental Neurotoxicity (DNT) Study in Rats for bifenthrin technical (MRID 46750501). A summary of a dietary feasibility and range-finding study (MRID 46750502) of bifenthrin technical in rats was included in the dose selection rationale and appendix to the data evaluation record for the DNT study. This DNT study was required as part of the conditional registration of bifenthrin. The action was successfully completed, and the conclusions of the study are reported here.

I. CONCLUSIONS

The Registration Action Branch I (RAB 1) has reviewed the Developmental Neurotoxicity Study in Rats (MRID 46750501) for bifenthrin technical. The study is classified as **Acceptable/NonGuideline** and may be used for regulatory purposes. It does not, however,

satisfy the guideline requirement for a developmental neurotoxicity study in rats (OPPTS 870.6300, §83-6); OECD 426 (draft) due to the pending review of the of positive control data. The submitted study satisfies the registration requirement of a Developmental Neurotoxicity Study in Rats.

II. STUDY REVIEWED

Developmental Neurotoxicity Study - Rat; OPPTS 870.6300

CITATION: Nemec, M. (2006). A dietary developmental neurotoxicity study of bifenthrin technical in rats. WIL Research Laboratories, LLC, Ashland, OH. Study number WIL-105021, January 13, 2006. MRID 46750501. Unpublished.

Nemec, M. (2006). A dietary feasibility and range-finding study of bifenthrin technical in rats. WIL Research Laboratories, LLC, Ashland, OH. Study number WIL-105019, January 13, 2006. MRID 46750502. Unpublished.

EXECUTIVE SUMMARY: In a developmental neurotoxicity study (MRID 46750501) Bifenthrin (94.8% a.i., lot PL02-0477) was administered in the diet to 25 female Crl:CD®(SD) rats per dose at dose levels of 0, 50, 100 and 125 ppm (0, 3.6, 7.2 and 9.0 mg/kg/day, respectively, during gestation; 0, 8.3, 16.2 and 20.7 mg/kg/day, respectively, during lactation) from gestation day (GD) 6 through lactation day (LD) 21. Dietary concentrations were selected on the basis of a range-finding study (MRID 46750502). A Functional Observational Battery (FOB) was performed on all dams on GDs 10 and 15 and on LDs 10 and 21. On postnatal day (PND) 4, litters were culled to yield four males and four females (as closely as possible). Offspring were allocated for detailed clinical observations (FOB) and assessment of motor activity, auditory startle reflex habituation, learning and memory (water maze testing) and neuropathology at termination (PND 72). On PND 21, the whole brain was collected from 10 pups/sex/group for micropathologic examination and morphometric analysis. Pup physical development was evaluated by body weight. The age of sexual maturation (vaginal opening in females and preputial separation in males) was assessed.

No dams died during the study. Maternal body weight, body weight gain and food consumption were unaffected by treatment. Tremors were observed during the daily examinations in 8/23 females at 100 ppm beginning on LD 14 and in 23/25 females in the 125 ppm group beginning on LD 4. In the 100 ppm group, the tremors were graded as slight and resolved in 4/8 females after one occurrence; slight tremors were observed in the remaining 4/8 females 3-7 times. In the 125 ppm group, the tremors were graded slight to moderate and continued on multiple occasions (2-18 consecutive days) during lactation. Piloerection was observed once or twice in 6/25 females at 125 ppm, primarily during LDs 14-17. During the FOB, the mean number of grooming counts was significantly increased in females at 100 and 125 ppm during gestation and lactation. At 125 ppm, slight piloerection was observed in 4/25 females on GD 15 and in 1/25 or 2/25 females on LDs 10 and 21. Clonic convulsions (limb tremors) and tremors were noted in 2/25 and 7/25 females, respectively, in the 125 ppm group on LD 10. On LD 21, the number of females with these findings was 10/25 and 13/25, respectively. Clonic convulsions (limb tremors) and tremors were noted in 2/23 and 3/23 females in the 100 ppm group, respectively, on LD 21. Reproductive performance was unaffected by treatment.

2

The maternal LOAEL for bifenthrin in rats was 100 ppm (7.2 mg/kg/day during gestation and 16.2 mg/kg/day during lactation) based on clinical signs of neurotoxicity (tremors, clonic convulsions, and increased grooming counts). The maternal NOAEL is 50 ppm (3.6 mg/kg/day during gestation and 8.3 mg/kg/day during lactation).

The mean number of delivered pups per dam, percentage of liveborn and stillborn pups and sex ratio on the day of birth were not affected by treatment. There was no treatment-related effect on offspring body weight or body weight gain. The mean day for reaching sexual maturation (vaginal opening in females and balanopreputial separation in males) was not affected by treatment. Two of 20 females in the 125 ppm group had tremors during the detailed physical examinations on PND 28. During the FOB, an increase in the incidence of tremors and clonic convulsions (limb tremors) was observed in males at 125 ppm on PND 21. A significant increase in mean grooming counts was noted in females at 100 and 125 ppm on PND 21. No treatment-related effects on motor activity, acoustic startle response, or learning and memory testing were observed. Brain weight, length, and width and macroscopic findings were not affected by treatment. Historical control data were not provided for several microscopic findings and are therefore requested. In brain morphometry, a slight increase (3.5%) in the height of the hemisphere (Level 1) that was observed at 125 ppm was not considered toxicologically significant.

The offspring LOAEL for bifenthrin in rats is 100 ppm (7.2 mg/kg/day during gestation and 16.2 mg/kg/day during lactation; maternal dose) based on clinical signs of neurotoxicity (increased grooming counts). The offspring NOAEL is 50 ppm (3.6 mg/kg/day during gestation and 8.3 mg/kg/day during lactation). Direct dosing to pups was *not performed*.

This study is classified as **Acceptable/Non-guideline** and may be used for regulatory purposes. It does not, however, satisfy the guideline requirement for a developmental neurotoxicity study in rats [OPPTS 870.6300, §83-6; OECD 426 (draft)] due to the pending review of the positive control data.

3

EPA Reviewer: Robert J. Mitkus, PhD **Signature:** Robert J. Mitkus
Registration Action Branch 1, Health Effects Division (7509P) **Date:** 8/7/06
Work Assignment Manager: P.V. Shah, PhD **Signature:** P.V. Shah
Registration Action Branch 1, Health Effects Division (7509P) **Date:** 8/29/06

Template version 02/06

TXR#: 0054120

DATA EVALUATION RECORD

STUDY TYPE: Developmental Neurotoxicity Study - Rat; OPPTS 870.6300 (§83-6); OECD 426 (draft)

PC CODE: 128825 **DP BARCODES:** D327148

TEST MATERIAL (PURITY): Bifenthrin (94.8% a.i.)

SYNONYMS: None

CITATION: Nemec, M. (2006). A dietary developmental neurotoxicity study of bifenthrin technical in rats. WIL Research Laboratories, LLC, Ashland, OH. Study number WIL-105021, January 13, 2006. MRID 46750501. Unpublished .

Nemec, M. (2006). A dietary feasibility and range-finding study of bifenthrin technical in rats. WIL Research Laboratories, LLC, Ashland, OH. Study number WIL-105019, January 13, 2006. MRID 46750502. Unpublished .

SPONSOR: FMC Corporation, Princeton, NJ

EXECUTIVE SUMMARY: In a developmental neurotoxicity study (MRID 46750501) Bifenthrin (94.8% a.i., lot PL02-0477) was administered in the diet to 25 female Crl:CD®(SD) rats per dose at dose levels of 0, 50, 100 and 125 ppm (0, 3.6, 7.2 and 9.0 mg/kg/day, respectively, during gestation; 0, 8.3, 16.2 and 20.7 mg/kg/day, respectively, during lactation) from gestation day (GD) 6 through lactation day (LD) 21. Dietary concentrations were selected on the basis of a range-finding study (MRID 46750502). A Functional Observational Battery (FOB) was performed on all dams on GDs 10 and 15 and on LDs 10 and 21. On postnatal day (PND) 4, litters were culled to yield four males and four females (as closely as possible). Offspring were allocated for detailed clinical observations (FOB) and assessment of motor activity, auditory startle reflex habituation, learning and memory (water maze testing) and neuropathology at termination (PND 72). On PND 21, the whole brain was collected from 10 pups/sex/group for micropathologic examination and morphometric analysis. Pup physical development was evaluated by body weight. The age of sexual maturation (vaginal opening in females and preputial separation in males) was assessed.

No dams died during the study. Maternal body weight, body weight gain and food consumption were unaffected by treatment. Tremors were observed during the daily examinations in 8/23 females at 100 ppm beginning on LD 14 and in 23/25 females in the 125 ppm group beginning on LD 4. In the 100 ppm group, the tremors were graded as slight and resolved in 4/8 females

5

after one occurrence; slight tremors were observed in the remaining 4/8 females 3-7 times. In the 125 ppm group, the tremors were graded slight to moderate and continued on multiple occasions (2-18 consecutive days) during lactation. Piloerection was observed once or twice in 6/25 females at 125 ppm, primarily during LDs 14-17. During the FOB, the mean number of grooming counts was significantly increased in females at 100 and 125 ppm during gestation and lactation. At 125 ppm, slight piloerection was observed in 4/25 females on GD 15 and in 1/25 or 2/25 females on LDs 10 and 21. Clonic convulsions (limb tremors) and tremors were noted in 2/25 and 7/25 females, respectively, in the 125 ppm group on LD 10. On LD 21, the number of females with these findings was 10/25 and 13/25, respectively. Clonic convulsions (limb tremors) and tremors were noted in 2/23 and 3/23 females in the 100 ppm group, respectively, on LD 21. Reproductive performance was unaffected by treatment.

The maternal LOAEL for bifenthrin in rats was 100 ppm (7.2 mg/kg/day during gestation and 16.2 mg/kg/day during lactation) based on clinical signs of neurotoxicity (tremors, clonic convulsions, and increased grooming counts). The maternal NOAEL is 50 ppm (3.6 mg/kg/day during gestation and 8.3 mg/kg/day during lactation).

The mean number of delivered pups per dam, percentage of liveborn and stillborn pups and sex ratio on the day of birth were not affected by treatment. There was no treatment-related effect on offspring body weight or body weight gain. The mean day for reaching sexual maturation (vaginal opening in females and balanopreputial separation in males) was not affected by treatment. Two of 20 females in the 125 ppm group had tremors during the detailed physical examinations on PND 28. During the FOB, an increase in the incidence of tremors and clonic convulsions (limb tremors) was observed in males at 125 ppm on PND 21. A significant increase in mean grooming counts was noted in females at 100 and 125 ppm on PND 21. No treatment-related effects on motor activity, acoustic startle response, or learning and memory testing were observed. Brain weight, length, and width and macroscopic findings were not affected by treatment. Historical control data were not provided for several microscopic findings and are therefore requested. In brain morphometry, a slight increase (3.5%) in the height of the hemisphere (Level 1) that was observed at 125 ppm was not considered toxicologically significant.

The offspring LOAEL for bifenthrin in rats is 100 ppm (7.2 mg/kg/day during gestation and 16.2 mg/kg/day during lactation; maternal dose) based on clinical signs of neurotoxicity (increased grooming counts). The offspring NOAEL is 50 ppm (3.6 mg/kg/day during gestation and 8.3 mg/kg/day during lactation). Direct dosing to pups was not performed.

This study is classified as **Acceptable/Non-guideline** and may be used for regulatory purposes. It does not, however, satisfy the guideline requirement for a developmental neurotoxicity study in rats [OPPTS 870.6300, §83-6; OECD 426 (draft)] due to the pending review of the positive control data.

COMPLIANCE: Signed and dated GLP, Data Confidentiality, and Flagging statements were provided. A Quality Assurance statement was not provided.

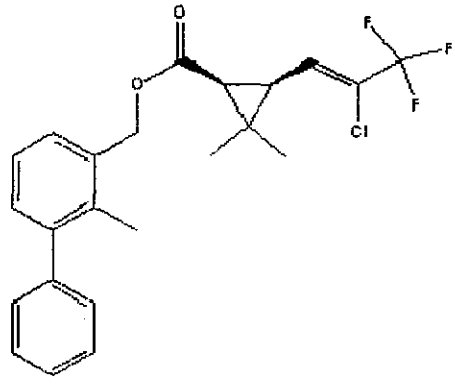
I. MATERIALS AND METHODS

A. MATERIALS:

1. **Test material:** Bifenthrin

Description:	Brown, very viscous liquid (after heating)
Lot #:	PL02-0477
Purity:	94.9% cis-bifenthrin and 0.64% trans-bifenthrin at beginning of study; 93.6% cis-bifenthrin and 0.62% trans-bifenthrin at end of study
Compound stability:	Expiration date: December 3, 2003 (chemical used past the expiration date but analyses showed the composition had not changed)
CAS # of TGAI:	82657-04-3

Structure:



2. **Vehicle:** Acetone

3. **Test animals (P):**

Species:	Rat
Strain:	Crl:CD®(SD)
Age at mating:	12 wks
Wt. on gestation day 0:	219-291 g
Source:	Charles River Laboratories, Inc., Raleigh, NC
Housing:	From GD 0 until LD 21 the pregnant animals and their litters were housed in plastic maternity cages; after weaning, litters housed together in plastic maternity cages; after PND 28, offspring housed individually in wire-mesh cages
Diet:	PMI Nutrition International, LLC, Certified Rodent LabDiet® 5002 *ad libitum*
Water:	Reverse osmosis-purified drinking water *ad libitum*
Environmental conditions:	Temperature: 71.2-72.6°F Humidity: 33.8-45.9% Air changes: 10/hour Photoperiod: 12 hrs dark/12 hrs light
Acclimation period:	Fourteen days

B. PROCEDURES AND STUDY DESIGN:

1. **In life dates:** Start: January 18, 2005; End: September 22, 2005

2. **Study schedule:** Maternal animals were mated with untreated males. The test substance was administered in the diet to the dams from gestation day (GD) 6 through lactation day (LD) 21.

Pups were weaned on PND 21, after which time maternal animals were killed. F_1 pups remained on study until either PND 21 or 72 (study termination).

3. **Mating procedure:** Females were placed in cages with untreated resident males from the same strain and source for mating. Mating was confirmed by detection of a vaginal plug or sperm in a vaginal smear (GD 0). Each pregnant female was placed into an individual cage with a solid bottom and bedding, where it was maintained through gestation and lactation.

4. **Animal assignment**: Mated females were assigned to groups using the laboratory's computer program which randomized the animals based on stratification of the GD 0 body weight in a block design. Offspring were randomly assigned to testing subgroups at the time of litter standardization on PND 4 (Table 1).

TABLE 1. Study design

Experimental parameter		Diet concentration (ppm)			
		0	50	100	125
Maternal animals					
No. of maternal animals assigned		25	25	25	25
FOB (GD 10, 15; LD 10, 21)		25	25	25	25
Offspring					
Subset A	FOB (PND 4, 11, 21, 35, 45, 60)	20/sex*	20/sex*	20/sex*	20/sex*
	Auditory startle (PND 20, 60)	20/sex*	20/sex*	20/sex*	20/sex*
	Locomotor activity (PND 13, 17, 21, 61)	20/sex*	20/sex*	20/sex*	20/sex*
	Learning and memory (PND 62)	20/sex*	20/sex*	20/sex*	20/sex*
	Brain weight, measurements, and macroscopic observations (PND 72)***	15/sex*	15/sex*	15/sex*	15/sex*
	Brain microscopic observations and Morphometry (PND 72)***	10/sex*	--	--	10/sex*
Subset B	Learning and memory (PND 25)**	20/sex	20/sex	20/sex	20/sex
Subset C	Brain weight, measurements, and macroscopic observations (PND 21)	15/sex	15/sex	15/sex	15/sex
	Brain microscopic observations and Morphometry (PND 21)	10/sex	--	--	10/sex

* Subset A offspring were used for all FOB, Auditory Startle, Motor Activity and PND 62 Learning and Memory Assessments. The same offspring were evaluated at multiple time points.
** Subset B offspring were tested only in the PND 25 Learning and Memory Assessment.
*** Animals used to evaluate brain weight, neuropathological and morphometric endpoints on PND 72 were randomly selected from animals previously allocated to Subset A behavioral testing.

5. **Dose selection rationale:** Dietary concentrations were selected based on the results of a preliminary dose range-finding study (MRID 46750502). Slight to moderate whole-body tremors were observed during lactation in 8/10 dams treated at 125 ppm. No effects were observed in offspring, although plasma levels of bifenthrin were comparable in maternal animals and their offspring. Mean offspring plasma levels were slightly lower on lactation day 22 than on lactation day 4; however, variability was quite large on lactation day 4 (range: 0.19-0.33 ppm) and lactation day 22 (range: 0.05-0.41 ppm). Mean levels of bifenthrin in maternal milk on lactation day 5 were 3.38, 5.05, 3.33, 5.11, and 8.20 at 50, 65, 80, 100, and 125 ppm. Mean levels peaked on lactation day 11 and were comparable on lactation day 17, relative to lactation day 5. Details of the study results are discussed in the Appendix of this DER.

6. **Dosage administration:** The test article was administered to maternal animals in the diet on GD 6 through LD 21. The test material was administered as a constant concentration (ppm) in the diet. Doses (mg/kg/day) administered during gestation and lactation were calculated from dietary concentrations, food consumption, and body weight data.

7. **Dosage preparation and analysis:** The test material was heated in an oven at approximately 80°C until a liquid was formed. The appropriate amount of bifenthrin for each test group was weighed into a tared weighing vessel with 20 mL of acetone. The test material was then added on a weight/weight basis into a Hobart mixing bowl with the appropriate amount of feed and mixed for 10 minutes. The premix was then mixed for 15 minutes with enough basal diet to achieve the total batch size of homogeneous diet. The control diet was prepared in the same manner without the test article. The prepared diets were placed in storage bags that remained open for 24 to 36 hours to allow the acetone to evaporate prior to administration. The test diets were prepared weekly and stored at room temperature.

   Stability testing was done during the range-finding study (MRID 46750502). Stability of the high and low concentration diets was established after 17 days of room temperature storage. Homogeneity was assessed during the definitive study, by collecting samples from the top, middle, and bottom strata of each formulation and from the middle stratum of the control formulation. Samples from the middle of the control and test diets were collected weekly throughout the administration period from all batches and kept at room temperature until analyzed for test article concentration.

   Results:

   Homogeneity analysis: The group mean (RSD%) for the 50, 100 and 125 ppm diets was 52.7 (1.8%), 105 (1.9%) and 130 (1.6%) ppm, respectively.

   Stability analysis: The percentage active ingredient for the 50 and 125 ppm formulations after 17 days at room temperature was 95.1% and 107%, respectively, of their initial measured concentration.

   Concentration analysis: The mean concentration (% of nominal) of the five samples for the 50, 100 and 125 ppm formulations was 48.1 (96.2%), 96.8 (96.8%) and 124 (99.5%) ppm, respectively.

   The analytical data indicated that the mixing procedure was adequate and that the difference between nominal and actual dosage to the study animals was acceptable.

C. OBSERVATIONS:

1. **In-life observations:**

   a. **Maternal animals**: Twice daily checks for mortality or moribundity and daily cage-side observations were conducted on maternal animals. Clinical observations of the dams were conducted daily. Signs of toxicity were recorded as they were observed.

All dams in each group were observed outside the home cage at least twice during gestation (GDs 10 and 15) and twice during lactation (LDs 10 and 21). Testing was performed by the same technicians, when possible, without knowledge of the group assignments. No details were provided on how the testing was conducted. The following functional observations were recorded:

	FUNCTIONAL OBSERVATIONS
X	Signs of autonomic function, including: 1) Ranking of degree of lacrimation and salivation, with range of severity scores from none to severe 2) Presence or absence of piloerection and exophthalmos, 3) Ranking or count of urination and defecation, including polyuria and diarrhea 4) Pupillary function such as constriction of the pupil in response to light, or a measure of pupil size 5) Degree of palpebral closure, e.g., ptosis.
X	Description, incidence, and severity of any convulsions, tremors, or abnormal movements.
X	Description and incidence of posture and gait abnormalities.
X	Description and incidence of any unusual or abnormal behaviors, excessive or repetitive actions (stereotypies), emaciation, dehydration, hypotonia or hypertonia, altered fur appearance, red or crusty deposits around the eyes, nose, or mouth, and any other observations that may facilitate interpretation of the data.

Individual maternal body weight was recorded on GDs 0, 6, 9, 12, 15 and 20 and on LDs 1, 4, 7, 11, 14, 17 and 21. Food consumption was determined on GDs 0, 6, 9, 12, 15 and 20 and on LDs 1, 4, 7, 11, 14, 17 and 21 and was reported as g/animal/day and g/kg/day. Mean compound consumption (mg/kg/day) was determined by dividing the concentration of the test material in the diet (mg/kg) by the g/kg/day food consumption value for each interval.

b. Offspring:

i. **Litter observations:** On the day of parturition, offspring were sexed and examined for gross malformations. The number of stillbirths and live offspring was recorded. The day of completion of parturition was designated as lactation day (postnatal day; PND) 0. Each litter was examined daily for survival and all deaths were recorded. Each offspring received a detailed physical examination on PND 4, 11 and 21 and at weekly intervals thereafter. Sex determination was also conducted on PND 4, 11 and 21. The pups were weighed on PND 1, 4, 7, 11, 13, 17 and 21 and at weekly intervals thereafter and whenever they were removed from their cages for behavioral testing.

On PND 4, litters were standardized using random procedures to a maximum of 8 pups/litter; excess pups were killed and discarded. Litters with fewer than six pups or that did not meet the sex ratio criteria (at least 3/sex) were not used for neurobehavioral or neuropathological evaluation.

ii. **Developmental landmarks**: Beginning on PND 35, male offspring were examined daily for balanopreputial separation. Beginning on PND 25, female offspring were examined daily for vaginal patency. The age of onset and body weight at attainment were recorded.

iii. **Postweaning observations:** After weaning on postnatal day 21, offspring received a detailed physical examination at weekly intervals. Individual offspring body weight data were recorded weekly.

iv. **Neurobehavioral evaluations**: Observations and the schedule for those observations are summarized as follows from the report.

a) **Functional observational battery (FOB):** On PNDs 4, 11, 21, 35, 45, and 60, twenty offspring/sex/group (Subset A) were examined outside the home cage in an FOB assessment, as appropriate for the developmental stage being observed. The same parameters assessed in the maternal FOB were examined in offspring, except that assessments on PNDs 21, 35, 45 and 60 included forelimb and hindlimb grip strength measurements. The same offspring were evaluated at each time point. Any animal found dead between PND 4 and 60 was replaced with another pup of the same age and sex. The technicians performing the testing were unaware of the animals' group assignments. No further details of the testing methods were given.

b) **Motor activity testing:** Motor activity was evaluated in 20 rats/sex/dose (Subset A) on PNDs 13, 17, 21, and 61. The same animals were tested at each interval, except for one male and one female at 50 ppm, each of which died on PND 19 and 15, respectively. The second female was found dead on PND 17 and so was replaced with a third female. Activity was measured using the SDI Photobeam Activity System (San Diego Instruments), which includes a series of infrared photobeams surrounding a clear, plastic rectangular cage. Square black plastic enclosures in turn surrounded the cages in order to reduce potential external visual stimuli. Data were collected in five-minute epochs over a test duration of 60 minutes. Data for ambulatory and total motor activity were recorded. Total motor activity was defined as a combination of fine motor skills (interruption of a single photobeam) and ambulatory motor activity (interruption of two or more consecutive photobeams).

c) **Auditory startle reflex habituation:** Auditory startle reflex habituation testing was performed on 20 offspring/sex/dose (Subset A) on PNDs 20 and 60, using the SR-Lab Startle Response System (San Diego Instruments). The same animals were tested at each interval. Each isolation chamber was composed of a wood core measuring 15x16x23 inches and covered with laboratory-grade plastic laminate. Each cabinet was equipped with an internal light, a fan, two viewing lenses and a white-noise generation system. The animal was placed in a cylindrical enclosure of appropriate size, which was then placed into the isolation cabinet. Each enclosure was equipped with a motion sensor.

Testing was performed in a room equipped with a white-noise generation system set to operate at 70±10 decibels (dB). Each test session consisted of a five-minute acclimation period with 65±5-dB broadband background white noise. The startle stimulus for each trial was a 115±5-dB mixed-frequency noise burst stimulus of approximately 20 milliseconds in duration. Responses were recorded during the first 100 milliseconds following the onset of the startle stimulus for each trial. Each session consisted of 50 trials with an eight-second intertrial interval. Startle response

measurements included maximum response amplitude (V_{MAX}), average response amplitude (V_{AVE}), and latency to V_{MAX} (T_{MAX}), which were analyzed in five blocks of 10 trials each.

d) **Learning and memory testing:** Learning and memory testing was performed on 20 offspring/sex/dose on PNDs 25 and 62 (Subsets B and A, respectively) using a water-filled, eight-unit T-maze similar to that described by Biel[1]. Animals were required to traverse the maze and escape by locating a platform hidden beneath the water surface. The amount of time required to escape and the number of errors were recorded. An error was defined as any instance when an animal deviated from the correct channel with all four feet.

The testing intervals consisted of three phases conducted over seven consecutive days. For phase one, which was performed on day one of the Biel maze procedure, animals were placed in a straight channel opposite the escape platform and the time required for each animal to escape was recorded. Each animal was given four trials to assess swimming ability and motivation to escape.

In phase two (evaluation of sequential learning), which was conducted on days 2-6 of the Biel maze procedure, animals were allowed two trials per day for two consecutive days to solve the maze via path A. Animals were then allowed two trials per day for three consecutive days to solve the path B maze (reverse of path A). For each trial, an animal was allowed three minutes to solve the maze; if it was not successful, the animal was placed on the escape platform for up to 20 seconds and then removed. The minimum intertrial interval was one hour.

In phase three, which was conducted on day 7 of the Biel maze procedure, memory was tested by challenging the animal to solve the maze in path A. Each animal was given two trials to solve the maze.

The maze data were reported as the mean time to escape per trial for each of the three phases (i.e., swimming ability and motivation, sequential learning, and memory). The mean number of errors per trial was also recorded for phases two and three.

2. **Postmortem observations:**

a. **Maternal animals:** Females that failed to meet the litter size or litter sex ratio (at least 3 pups/sex) on LD 4 were euthanized by carbon dioxide inhalation and subjected to gross necropsy. The necropsy consisted of examination of the external surface, all orifices and the cranial, thoracic, abdominal and pelvic cavities, including viscera. The number and location of implantation sites were recorded.

All surviving females with viable offspring on LD 21, those with total litter loss, and those that did not deliver were euthanized with carbon dioxide inhalation. Gross

[1] Biel, W.C. (1940) Early age differences in maze performance in the albino rat. J. Genet. Psych. 56:439-453.

necropsy was performed as described in the above paragraph, except for the cranial cavities. In females that delivered, the number of former implantation sites was recorded. Pregnancy status was determined for females that failed to deliver. Uteri with no evidence of implantation were opened and placed in a 10% ammonium sulfide solution for detection of early implantation loss. Tissues from all maternal animals were preserved in 10% neutral-buffered formalin for possible histopathologic examination.

b. **Offspring**: Intact offspring dying or euthanized *in extremis* (by intraperitoneal injection of sodium pentobarbital) from PND 0 to 4 were necropsied using a fresh dissection technique that included the heart and major vessels. The stomach was examined for the presence of milk on PND 0 and 1. A detailed gross necropsy was conducted on any pup dying after PND 4 and tissues were saved for possible histological examination, as deemed necessary by gross findings. Culled offspring were weighed, euthanized by intraperitoneal injection of sodium pentobarbital on PND 4 and discarded without examination.

Offspring not selected for behavioral or neuropathological evaluations were euthanized by carbon dioxide inhalation on PND 21 and subjected to gross necropsy. Tissues were retained only if deemed necessary by the gross findings, and the carcass was discarded.

Offspring scheduled for euthanasia after completion of the PND 25 learning and memory testing (Subset B) were euthanized by CO_2 inhalation and subjected to gross necropsy. Offspring in Subset A not allocated for neuropathology on PND 72 were euthanized by carbon dioxide inhalation and subjected to a gross necropsy examination.

On PND 21, one male and/or one female offspring from each litter (Subset C, 15/sex/group) was examined for neuropathology. All animals were perfused *in situ* with 4% paraformaldehyde/1.4% glutaraldehyde. The whole brain (including olfactory bulbs) was removed, weighed and the size (length and width) recorded. Abnormal coloration or lesions of the brain and spinal cord were recorded. All brains were prepared for histopathological examination by embedding in paraffin, sectioning and staining with hematoxylin and eosin. Sections from all major brain regions (olfactory bulbs, cerebral cortex, hippocampus, basal ganglia, thalamus, hypothalamus, midbrain, pons, medulla oblongata and cerebellum) were examined microscopically and morphometrically from 10 rats/sex in the control and high dose groups. Morphometric measurements were made on Levels 1, 3 and 5 of the brain. Pair-wise matching between measured groups was performed. Level 1 was a coronal section of rostral cerebrum, including caudoputamen. Level 3 was a coronal section of mid-cerebrum (cerebral cortex, hippocampal formation, thalamus, etc.). Level 5 was a mid-sagittal section of cerebellum and pons. Measurements of Level 5 were not paired since the other half of these tissues were sectioned transversely for visualization of the cerebellar nuclei. Measurements were made on homologous sections to ensure that dimensions of the regions were comparable. If there was a deformation or irregularity of a region, morphometric analyses could not be performed on certain individual animals. An explanation was not provided as to what was believed to be the cause of a deformation or irregularity.

On PND 72, one male and/or one female from each litter (15/sex/group) were randomly selected from the pups involved in neurobehavioral testing and were euthanized by carbon dioxide inhalation and perfused as those on PND 21. The whole brain (including olfactory bulb) was removed, weighed and the size (width and length) recorded. The central nervous system and the peripheral nervous tissues were embedded in paraffin and plastic, respectively. Tissues were sectioned and stained with hematoxylin and eosin. Morphometric measurements were made on Levels 1, 3 and 5 of the brain, as described for PND 21. If there was a deformation or irregularity of a region, morphometric analyses could not be performed on certain individual animals. An explanation was not provided as to what was believed to be the cause of a deformation or irregularity. The following tissues from control and high-dose animals perfused *in situ* at study termination (PND 72) were examined microscopically:

The CHECKED (X) tissues were evaluated for adult offspring.

X	CENTRAL NERVOUS SYSTEM	X	PERIPHERAL NERVOUS SYSTEM
	BRAIN	X	**SCIATIC NERVE**
X	Forebrain (olfactory bulbs)		Mid-thigh
X	Center of cerebrum		Sciatic notch
X	Midbrain		
X	Cerebellum	X	**OTHER**
X	Pons	X	Sural nerve
X	Medulla oblongata	X	Tibial nerve
X	**SPINAL CORD**	X	Peroneal nerve
X	Cervical swelling	X	Lumbar dorsal root fibers
X	Lumbar swelling	X	Lumbar dorsal root ganglion
X	**OTHER**	X	Lumbar ventral root fibers
	Gasserian ganglion	X	Cervical dorsal root ganglion
X	Trigeminal nerves	X	Cervical dorsal root fibers
X	Optic nerve	X	Cervical ventral root fibers
X	Eyes		

D. DATA ANALYSIS:

1. **Statistical analyses**: Two-tailed, sex-specific comparisons of each treated group with the control group were conducted using a minimum significance level of 0.05. The following parameters were subjected to a parametric one-way Analysis of Variance (ANOVA) to determine intergroup differences: mean maternal and offspring body weight and body weight gain, maternal food consumption, length of gestation, implantation sites, unaccounted sites, number of pups born, live litter size, day of acquisition of balanopreputial separation or vaginal patency and body weight on day of acquisition, brain weight, brain dimensions of F_1 pups, brain morphometric data, continuous FOB data, and Biel maze straight channel data. If the ANOVA revealed statistically significant ($p<0.05$) intergroup variance, Dunnett's test was used to compare the treated groups to the control group. Mean litter proportions (percent per litter) of pup viability and males per litter were subjected to the Kruskal-Wallis nonparametric ANOVA to determine intergroup differences. If the ANOVA revealed statistically significant intergroup

variance, Dunn's test was used to compare the treated and control groups. FOB data which yielded scalar and descriptive data were analyzed using the Fisher's Exact Test.

Analysis of behavioral data was conducted by BioSTAT Consultants, Inc. A repeated measures ANOVA (RANOVA) was used to analyze intrasession total and ambulatory counts from motor activity testing, V_{MAX} and T_{MAX} values from the acoustic startle test, and time to escape and number of errors from the learning and memory assessments. In learning and memory analysis, the time to escape for an animal that did not escape the maze in the allotted time was set equal to the maximum allotted time. For motor activity, factors in the model included treatment (TRT), time interval (TIME) and the interaction of time interval and treatment group (TRT*TIME). For acoustic startle and learning and memory, factors in the model included treatment group (TRT), trial or trial block (TRIAL), and the interaction of trial block and treatment group (TRT*TRIAL). The SAS procedure PROC-MIXED was used for the analysis with the random effect of animal included as the repeated measurement. The covariance structure across time was selected by comparing Akaike's Information Criterion for first-order autoregressive homogeneous and compound symmetric structures.

A monotonic dose-response relationship was evaluated using sequential linear trend tests based on ordinal spacing of dose levels. The linear dose by time interaction (Lin Trt*Time) for motor activity and the linear dose by trial interaction (Lin Trt*Trial) for acoustic startle response and learning and memory were evaluated and, if significant at the 0.05 level, trend tests on treatment means were performed at the 0.05 level for each time interval. If the linear dose by time (or trial) interaction was not significant, the trend test was conducted across the pooled time intervals of the entire session only.

Non-monotonic dose responses were evaluated whenever no significant linear trends were detected but TRT and/or TRT*TIME (for motor activity) and/or TRT*TRIAL (for auditory startle response and learning and memory) interaction was significant at the 0.01 level. Within the framework of the RANOVA, pair-wise comparisons were made for each individual treated group with the control group through linear contrasts. If the TRT*TIME or TRT*TRIAL interaction was significant, the comparisons were conducted for each interval. If only the TRT effect was significant, the comparisons were conducted across the pooled intervals. These nonmonotonic dose response comparisons were conducted at the 0.01 significance level. It was not stated why a more stringent statistical test was performed (α=0.01) for non-monotonic dose responses than for monotonic dose-response relationships.

2. Indices:

a. Reproductive indices (calculated by reviewer):

$$\text{Fertility index (\%)} = \frac{\text{number of females pregnant}}{\text{number of females mated}} \times 100\%$$

$$\text{Gestation index (\%)} = \frac{\text{number of females with live pups on the day of birth}}{} \times 100\%$$

15

number of females pregnant

b. **Offspring viability indices:** The following viability (survival) indices were calculated:

$$\text{Mean Live Litter Size} = \frac{\text{Total number of viable pups on PND 0}}{\text{No. of litters with viable pups on PND 0}}$$

$$\text{Postnatal Survival Between Birth and PND 4 (\% per litter)} = \frac{\Sigma\ (\text{Viable pups per litter on PND 4} / \text{No. of pups born per litter})}{\text{No. of litters per group}} \times 100\%$$

$$\text{Postnatal Survival for All Other Intervals (\% Per Litter)} = \frac{\Sigma\ (\text{Viable pups per litter at end of interval N} / \text{No. viable pups per litter at start of interval N})}{\text{No. of litters per group}} \times 100\%,$$

where N= PNDs 0-1, 1-4 (pre-selection), 4 (post-selection)-7, 7-14, 14-21, or 4 (post selection)-21

3. **Positive and historical control data:** No positive control data were submitted with this study, although several method development/validation studies, in which various neurotoxicants were used, were included in the study report. Positive control data have been submitted to the Agency and are currently under review. Historical control data for FOB, motor activity, startle response, Biel maze and brain morphometry were included with the study report.

II. RESULTS

A. PARENTAL ANIMALS:

1. **Mortality and clinical and functional observations:** No deaths were reported in dams during gestation and lactation. Tremors were observed during the daily examination in 8/25 females at 100 ppm beginning on LD 14 (following 29-30 days of treatment) and in 23/25 females at 125 ppm beginning on LD 4 (following 19-20 days of treatment). In the 100 ppm group, the tremors were graded as slight and resolved in 4/8 females after one occurrence (LD 16 or 20); slight tremors were observed in the remaining 4/8 females on 3-7 days (generally consecutive) between LDs 14 and 21. In the 125 ppm group, the tremors were graded slight to moderate and continued on multiple occasions (2-18 consecutive days) during lactation. Piloerection was observed once or twice in 6/25 females at 125 ppm, primarily during LDs 14-17. The incidences of these clinical observations are presented in Table 2.

TABLE 2. Clinical observations (total occurrence/number of animals affected) in F_0 females [a]				
Observation	Diet concentration (ppm)			
	0	50	100	125
Lactation (n=25)				
Tremors	0/0	0/0	23/8	216/23
Piloerection	0/0	0/0	0/0	7/6

[a] Data obtained from page 156, MRID 46750501

During the FOB, the mean number of grooming counts was significantly increased in females at 125 ppm on GD 10 and LDs 10 and 21. An increase in mean grooming counts was also observed in females at 100 ppm on GD 10. The increase was supported by examination of the individual animal data. At 125 ppm, slight piloerection was observed in 4/25 females on GD 15 and in 1-2/25 females on LDs 10 and 21. Clonic convulsions (limb tremors) and tremors were noted in 2/25 and 7/25 females, respectively, in the 125 ppm group on LD 10. On LD 21, the number of females with clonic convulsions (limb tremors) and tremors at 125 ppm increased to 10/25 and 13/25, respectively. Clonic convulsions and tremors were noted in 2/23 and 3/23 females, respectively, in the 100 ppm group on LD 21. Increased incidences of clinical observations at 100 ppm on LD 21 were considered treatment-related, since neither convulsions nor tremors were observed in controls on that day. FOB data are presented in Table 3.

TABLE 3. FOB observations in F_0 females [a]				
Observation	Diet concentration (ppm)			
	0	50	100	125
Gestation				
Gestation day 10 (n=23-25)				
Grooming counts (mean ± S.D.)	0.2 ± 0.6	0.4 ± 0.9	0.9 ± 1.4	1.0* ± 1.1
Gestation Day 15				
Piloerection - slight (# affected/total)	0/24	0/24	0/23	4/25
Lactation				
Lactation day 10				
Piloerection – slight (# affected/total)	0/23	0/22	0/23	2/25
Convulsions – clonic (limb tremors) (# affected/total)	0/23	0/22	0/23	2/25
Tremors – slight (# affected/total)	0/23	0/22	0/23	7/25**
Grooming counts (mean ± S.D.) (n=22-25)	0.1 ± 0.3	0.4 ± 0.9	0.4 ± 1.0	0.9 ± 1.5*
Lactation day 21				
Piloerection – slight (#affected/total)	0/23	0/21	0/23	1/25
Convulsions – clonic (limb tremors) (# affected/total)	0/23	0/21	2/23	10/25**
Tremors – slight (# affected/total)	0/23	0/21	3/23	13/25**
Grooming counts (mean ± S.D.) (n=21-25)	0.0 ± 0.2	0.2 ± 0.6	0.3 ± 0.7	0.9 ± 1.2**

[a] Data obtained from pages 158-178, MRID 46750501.
* Statistically different ($p < 0.05$) from the control.
** Statistically different ($p < 0.01$) from the control.

2. **Body weight and food consumption:** Selected group mean body weight, body weight gain and food consumption values for pregnant or nursing dams are summarized in Table 4. A 23% ($P<0.05$) decrease in mean body weight gain in the 125 ppm group was observed during GD 12-15 only. Observation of the individual animal data indicated that the decrease was due to 2/25 animals (clear outliers) at 125 ppm that gained only 2 or 7 grams each during this

17

interval. In addition, the range of mean body weight change during GD 12-15 in the dose-range finding study (MRID 46750502) was 17-20 g across dose (including controls); dose-response was lacking. No treatment-related changes in body weight or food consumption were observed at any other interval during gestation or lactation.

TABLE 4. Mean (±SD) maternal body weight, body weight gain and food consumption [a]

Observations/study week	Diet concentration (ppm)			
	0	50	100	125
Gestation (n=23-25)				
Mean body weight (g) Gestation day 0	254 ± 16.4	250 ± 15.7	250 ± 11.7	252 ± 12.9
Mean body weight (g) Gestation day 6	290 ± 20.6	284 ± 18.0	282 ± 14.0	283 ± 15.8
Mean body weight (g) Gestation day 20	407 ± 34.0	405 ± 22.4	401 ± 25.6	398 ± 22.1
Mean weight gain (g) Gestation days 12-15	22 ± 5.2	21 ± 6.0	20 ± 5.8	17 ± 6.5* (23)
Mean weight gain (g) Gestation days 6-20	117 ± 19.6	121 ± 9.7	119 ± 15.9	115 ± 15.2
Mean food consumption (g/animal/day) Gestation days 6-20	24 ± 2.6	24 ± 1.5	23 ± 1.9	23 ± 1.5
Lactation (n=20-25)				
Mean body weight (g) Lactation day 1	300 ± 21.6	301 ± 18.1	290 ± 19.9	295 ± 16.9
Mean body weight (g) Lactation day 7	330 ± 27.3	324 ± 20.0	312 ± 19.9	322 ± 20.9
Mean body weight (g) Lactation day 21	342 ± 26.1	341 ± 23.3	333 ± 20.3	327 ± 23.1
Mean weight gain (g) Lactation days 1-21	43 ± 22.1	40 ± 17.7	42 ± 21.1	32 ± 19.1
Mean food consumption (g/animal/day) Lactation days 1-21	54 ± 4.6	55 ± 3.9	52 ± 3.7	54 ± 3.6

[a] Data obtained from pages 179-187, MRID 46750501.
% change relative to control in parentheses

3. **Maternal test substance intake:** Calculated from maternal food consumption and body weight data, test substance intake, expressed as mean daily mg test substance/kg body weight during gestation and lactation, is presented for each dose group in Table 5.

TABLE 5. Mean maternal test substance intake (mg/kg body weight/day) [a]

Period	Diet concentration (ppm)		
	50	100	125
Gestation			
Gestation days 6-20	3.6 ± 0.1	7.2 ± 0.4	9.0 ± 0.4
Lactation			
Lactation days 1-21	8.3 ± 0.6	16.2 ± 1.3	20.7 ± 1.3

[a] Data obtained from pages 183 and 190, MRID 46750501.

4. **Reproductive performance:** No treatment-related effects were observed on any reproductive performance parameter, including fertility index, gestation index, or gestation length (Table 6).

TABLE 6. Reproductive performance [a]				
Observation	Diet concentration (ppm)			
	0	50	100	125
Number mated	25	25	25	25
Number of litters	24	24	23	25
Intercurrent deaths	0	0	0	0
Fertility index (%)[b]	96	96	92	100
Gestation index (%)[b]	100	100	100	100
Mean (±SD) gestation duration (days)	21.7 ± 0.6	21.8 ± 0.4	21.6 ± 0.5	21.6 ± 0.5
Incidence of dystocia	0	0	0	0

[a] Data obtained from pages 191-192, MRID 46750501.

[b] Calculated by the reviewer.

5. **Maternal postmortem results**: No treatment-related changes were observed at gross necropsy. The mean number of implantation sites in the uterus was unaffected by treatment.

B. OFFSPRING:

1. **Viability and clinical signs**: Litter size and viability (survival) results from pups during lactation are summarized in Table 7. The mean number of delivered pups per dam and the percentage of liveborn and stillborn pups were not affected by treatment. The variability (SD=21.4) in the percentage of litter survival from PND 4-21 at 50 ppm was not apparent when the individual animal data were examined. There was no treatment-related effect on sex ratio on the day of birth. At 125 ppm, 2/20 female offspring had slight tremors during the detailed physical examinations conducted on PND 28, which was seven days after the last exposure of the dams to the test diet.

TABLE 7. Litter size and viability [a]

Observation	Diet concentration (ppm)			
	0	50	100	125
Number of litters	24	24	23	25
Number dams with liveborn litters	24	24	23	25
Number dams with total litter loss	0	1	0	0
Total number pups born[b]	385	377	386	380
Number born dead/missing	2	1	8	2
Number dams with stillborn pups[b]	2	1	6	2
Total number liveborn pups	383	376	378	378
Mean no. pups born/dam	16.0 ± 2.5	15.7 ± 1.4	16.8 ± 1.9	15.2 ± 2.1
Mean live litter size (PND 0)	16.0 ± 2.6	15.7 ± 1.5	16.4 ± 2.0	15.1 ± 2.2
Sex ratio (% males)	49.7 ± 13.3	49.6 ± 13.8	49.8 ± 12.7	49.4 ± 13.1
Percentage of litter survival				
PND 0 [c]	99.4 ± 1.9	99.7 ± 1.5	98.0 ± 4.2	99.4 ± 2.0
PND 0 - PND 1	99.1 ± 2.1	100.0 ± 0.0	98.7 ± 3.7	100.0 ± 0.0
PND 1 - PND 4 (pre-selection)	99.0 ± 2.2	99.5 ± 1.8	98.0 ± 4.3	99.1 ± 2.5
PND 4 (post-selection) - PND 7	98.9 ± 3.6	100.0 ± 0.0	98.9 ± 3.6	100.0 ± 0.0
PND 7 - PND 14	100.0 ± 0.0	99.4 ± 2.7	100.0 ± 0.0	99.5 ± 2.5
PND 14 - PND 21	100.0 ± 0.0	95.5 ± 21.3	100.0 ± 0.0	100.0 ± 0.0
Birth - PND 4 (pre-selection)	97.6 ± 3.3	99.2 ± 2.3	94.8 ± 6.4	98.6 ± 3.8
PND 4 (post-selection) - PND 21	98.9 ± 3.6	94.9 ± 21.4 [d]	98.9 ± 3.6	99.5 ± 2.5

[a] Data obtained from pages 192, 195, 197-199, and 675-678, MRID 46750501.
[b] Calculated by the reviewer using data from pages 675-678, MRID 46750501.
[c] Relative to number born
[d] Variability not apparent in individual animal data.

2. **Body weight**: No treatment-related effects on offspring body weight during lactation were observed. Decreases in pre-weaning mean body weight and body weight gain were observed in males and females at 100 ppm (Table 8). However, these statistically significant changes were not considered toxicologically significant, since the magnitude was small (<10%) and dose response was lacking. Selected mean pre-weaning pup body weight data are presented in Table 8.

TABLE 8. Mean (±SD) pre-weaning pup body weight and body weight gain (n=21-25)[a]								
Postnatal day	Diet concentration (ppm)							
	0	50	100	125	0	50	100	125
	Males				Females			
Body Weight (g)								
1	6.9 ± 0.7	6.9 ± 0.6	6.5 ± 0.5	6.7 ± 0.5	6.5 ± 0.6	6.5 ± 0.6	6.1 ± 0.5	6.4 ± 0.4
4[b]	9.3 ± 1.2	9.3 ± 0.7	8.6 ± 0.7*	9.3 ± 0.9	8.8 ± 1.1	8.8 ± 0.6	8.3 ± 0.8*	9.0 ± 0.8
7	15.1 ± 1.7	14.9 ± 1.5	14.0 ± 1.2*	15.2 ± 1.5	14.5 ± 1.6	14.1 ± 1.6	13.3 ± 1.4*	14.8 ± 1.3
13	28.1 ± 2.4	27.7 ± 3.4	25.9± 2.1*	28.5 ± 2.4	27.3 ± 2.7	26.5 ± 3.5	24.8 ± 2.3**	27.8 ± 2.4
21	49.6 ± 5.1	49.9 ± 4.5	45.6± 3.8*	49.0 ± 4.8	48.1 ± 5.7	47.7 ± 4.0	43.8 ± 3.9**	47.4 ± 5.0
Body Weight Gain (g)								
1-4	2.4 ± 0.7	2.5 ± 0.4	2.2 ± 0.4	2.6 ± 0.5	2.4 ± 0.7	2.3 ± 0.3	2.1 ± 0.4	2.6 ± 0.5
4-7	5.8 ± 0.7	5.7 ± 1.2	5.3 ± 0.8	5.9 ± 0.9	5.6 ± 0.7	5.4 ± 1.3	5.0 ± 0.9	5.8 ± 0.7
17-21	12.5 ± 2.7	12.0 ± 2.7	10.5 ± 2.1*	11.0 ± 2.0	12.1 ± 3.1	11.3 ± 2.2	10.0 ± 2.0*	10.7 ± 2.3

[a] Data obtained from pages 201-205, MRID 46750501.
[b] Before standardization (culling).
* Significantly different from control value, $p<0.05$.
** Significantly different from control value, $p<0.01$.

Decreases in mean post-weaning body weight were observed in males and females (statistically significant) at 100 ppm. However, the changes were not considered toxicologically significant, since the magnitude was small (<10%) and dose response was lacking. No changes in mean body weight gain were observed at any dose. Selected mean post-weaning offspring body weight data are presented in Table 9.

TABLE 9. Mean (±SD) post-weaning pup body weight (g)[a]								
PND	Diet concentration (ppm)							
	0	50	100	125	0	50	100	125
	Males				Females			
28	84 ± 7.1	82 ± 9.3	80 ± 5.8	83 ± 7.5	77 ± 8.4	79 ± 6.0	72 ± 6.4*	77 ± 7.0
35	147 ± 13.7	145 ± 15.7	139 ± 8.1	145 ± 11.5	127 ±11.9	128 ± 9.7	118 ±10.5*	125 ±9.8
56	330 ± 19.2	331 ± 23.0	322 ± 21.2	324 ± 18.2	213 ±19.3	216 ±17.6	206 ± 18.6	211 ± 13.6
72	402 ± 26.6	406 ± 27.4	399 ± 23.9	401 ± 25.3	250 ±18.4	252 ±20.1	241 ± 23.6	249 ± 13.8

[a] Data obtained from pages 214-217, MRID 46750501.
* Significantly different from control value, $p<0.05$.

3. Developmental landmarks:

a. Sexual maturation: No treatment-related effects on vaginal opening or balanopreputial separation were observed. The mean number of days to reach vaginal opening was 32.6, 32.2, 32.3 and 33.0 days for the 0, 50, 100 and 125 ppm groups, respectively. The mean number of days to balanopreputial separation was 44.8, 44.4, 45.2 and 44.8 days for the respective groups. Mean body weight on the day that criterion was reached was similar between the control and test groups for males and females. A 10% decrease was

21

observed in females at 100 ppm; however, the change was not dose-dependent, and no change in time to reach vaginal patency was observed at this dose. Sexual maturation data are presented in Table 10.

TABLE 10. Mean (±SD) age and body weight at sexual maturation [a]

Parameter	Diet concentration (ppm)			
	0	50	100	125
N (M/F)	20/20	20/20	20/20	20/20
Males				
Preputial separation (days)	44.8 ± 2.0	44.4 ± 1.8	45.2 ± 2.0	44.8 ± 2.6
Body weight (g)	232.8 ± 18.7	230.0 ± 21.8	229.5 ± 15.9	232.0 ± 20.7
Females				
Vaginal opening (days)	32.6 ± 1.1	32.2 ± 1.3	32.3 ± 0.9	33.0 ± 1.0
Body weight (g)	110.4 ± 10.9	107.5 ± 8.7	99.6 ± 10.9**	111.3 ± 10.8

[a] Data obtained from pages 208-209, MRID 46750501.
** Significantly different from control value, $p<0.01$.

4. Behavioral assessments:

a. **Functional observational battery:** An increase in the incidence of tremors and clonic convulsions (limb tremors) was observed in males at 125 ppm on PND 21 (4/20 vs. 0/20 in controls). A statistically significant increase in mean grooming counts was noted in females at 100 and 125 ppm on PND 21. No treatment-related changes in forelimb and hindlimb grip strength were observed. Incidences of FOB data are presented in Table 11.

TABLE 11. Incidence of FOB observations (# affected/total) on PND 21 in F_1 animals [a]

Observation	Diet concentration (ppm)			
	0	50	100	125
Males				
Convulsions – Clonic Slight	0/20	0/20	0/20	4/20
Tremors Slight	0/20	0/20	0/20	4/20
Females				
Grooming counts Mean ± S.D.	0.4 ± 0.8	0.4 ± 0.7	1.8 ± 1.4**	1.6 ± 1.6**

[a] Data obtained from pages 244 and 249, MRID 46750501.
** Statistically different ($p < 0.01$) from the control.

Motor activity: Total motor activity data are presented in Table 12a, and interval data for males and females are found in Tables 12b and 12c, respectively. Total ambulatory activity data are presented in Table 12d, while interval data for males and females are in Tables 12e and 12f, respectively. No treatment-related effects were observed on motor activity at any time point. A dose-dependent decrease (not statistically significant) in mean cumulative motor activity, relative to controls, was observed in PND 21 males;

however, mean cumulative motor activity in control animals did not appear to follow the general pattern of peaking on PND 17, then decreasing on PND 21[2].

TABLE 12a. Mean motor activity data [cumulative counts ± S.D. per session] [a]				
Test day	Diet concentration (ppm)			
	0	50	100	125
Males				
PND 13	440.65 ± 300.79	415.65 ± 137.75	535.30 ± 382.78	442.55 ± 474.14
PND 17	738.80 ± 597.88	1253.10 ± 902.36	835.55 ± 693.05	967.35 ± 681.28
PND 21	855.45 ± 362.38	653.30 ± 218.13	626.90 ± 265.95	605.80 ± 327.80
PND 61	2351.15 ± 637.87	2382.95 ± 510.09	2411.90 ± 581.56	2637.05 ± 543.55
Females				
PND 13	569.60 ± 549.85	390.95 ± 363.06	431.65 ± 221.43	384.30 ± 238.04
PND 17	710.70 ± 531.83	862.29 ± 677.22	912.75 ± 487.24	807.80 ± 443.83
PND 21	673.75 ± 374.80	699.40 ± 383.85	773.50 ± 451.25	634.55 ± 339.72
PND 61	2341.65 ± 503.59	2484.25 ± 761.26	2370.30 ± 894.41	2651.65 ± 686.63

[a] Data obtained from pages 281-296, MRID 46750501.
N = 20

TABLE 12b. Motor activity sub-sessions - males [counts ± S.D.] [a]					
Sub-session		Diet concentration (ppm)			
		0	50	100	125
PND 13	0-15	199.30 ± 198.84	149.80 ± 97.45	163.20 ± 101.46	150.75 ± 94.91
	16-30	80.25 ± 103.36	92.05 ± 70.48	134.45 ± 133.27	82.75 ± 141.94
	31-45	84.65 ± 101.76	75.50 ± 48.40	126.95 ± 120.49	105.00 ± 193.27
	46-60	76.45 ± 95.84	98.25 ± 61.85	110.70 ± 115.12	104.05 ± 137.85
PND 17	0-15	351.20 ± 205.09	487.10 ± 201.12	368.55 ± 240.51	425.95 ± 272.42
	16-30	142.60 ± 171.90	309.85 ± 270.92	202.25 ± 184.92	219.80 ± 197.52
	31-45	126.00 ± 184.20	245.45 ± 303.90	141.55 ± 191.16	139.35 ± 173.76
	46-60	119.00 ± 204.73	210.70 ± 274.31	123.20 ± 173.66	182.25 ± 212.93
PND 21	0-15	522.80 ± 121.68	485.60 ± 128.53	401.20 ± 151.84	438.90 ± 167.44
	16-30	168.60 ± 150.15	97.00 ± 94.10	118.90 ± 125.13	111.25 ± 115.77
	31-45	95.65 ± 118.51	53.75 ± 86.35	58.60 ± 97.17	30.45 ± 58.84
	46-60	68.40 ± 140.55	16.95 ± 18.28	48.20 ± 98.49	25.20 ± 68.54
PND 61	0-15	1126.75 ± 218.86	1184.80 ± 246.95	1114.10 ± 191.32	1183.60 ± 166.72
	16-30	534.35 ± 186.80	561.95 ± 177.56	535.10 ± 191.20	615.15 ± 150.40
	31-45	352.75 ± 194.76	374.35 ± 184.35	462.05 ± 197.37	455.55 ± 171.01
	46-60	337.30 ± 257.71	261.85 ± 184.63	300.65 ± 213.82	382.75 ± 267.82

[a] Data obtained from pages 281-296, MRID 46750501.
N = 20
* Significantly different from control value, $p<0.05$.

[2] Ruppert PH, Dean KF, Reiter LW. (1984). Development of locomotor activity of rat pups in figure-eight mazes. Dev Psychobiol 18(3):247-260.

TABLE 12c. Motor activity sub-sessions - females [counts ± S.D.] [a]					
Sub-session		Diet concentration (ppm)			
		0	50	100	125
PND 13	0-15	178.55 ± 177.92	125.65 ± 98.75	132.90 ± 77.37	136.30 ± 138.24
	16-30	162.35 ± 223.68	89.90 ± 76.00	95.65 ± 72.77	68.70 ± 58.44
	31-45	128.40 ± 162.69	78.85 ± 103.50	106.60 ± 91.00	72.55 ± 70.87
	46-60	100.30 ± 102.08	96.55 ± 145.70	96.50 ± 56.50	106.75 ± 102.57
PND 17	0-15	339.00 ± 182.65	355.33 ± 226.87	432.40 ± 185.66	344.25 ± 140.52
	16-30	178.40 ± 177.12	170.33 ± 164.08	227.30 ± 174.05	119.05 ± 131.54
	31-45	124.90 ± 156.39	160.81 ± 196.74	132.50 ± 150.19	150.05 ± 164.54
	46-60	68.40 ± 125.15	175.81 ± 245.69	120.55 ± 172.79	194.45 ± 201.77
PND 21	0-15	428.75 ± 117.54	447.60 ± 134.05	482.80 ± 128.53	440.40 ± 159.05
	16-30	111.65 ± 120.55	125.60 ± 141.78	122.30 ± 140.07	105.30 ± 108.02
	31-45	97.15 ± 136.44	80.30 ± 113.58	98.00 ± 160.17	57.35 ± 80.29
	46-60	36.20 ± 74.78	45.90 ± 86.50	70.40 ± 99.32	31.50 ± 75.42
PND 61	0-15	1043.85 ± 125.22	1090.05 ± 186.89	1049.75 ± 170.63	1095.25 ± 174.45
	16-30	548.90 ± 212.59	625.85 ± 268.63	530.45 ± 265.38	681.00 ± 192.97
	31-45	375.00 ± 222.16	433.70 ± 275.93	438.15 ± 306.93	473.35 ± 215.22
	46-60	373.90 ± 220.16	334.65 ± 242.66	351.95 ± 326.20	402.05 ± 271.05

[a] Data obtained from pages 281-296, MRID 46750501.
N = 20

TABLE 12d. Ambulatory activity data – mean (±S.D.) number of total counts per session [a]				
Test Day	Diet concentration (ppm)			
	0	50	100	125
Males				
PND 13	118.30 ± 160.74	57.30 ± 66.40	138.55 ± 227.25	129.50 ± 289.29
PND 17	261.70 ± 251.21	467.90 ± 396.30	299.70 ± 306.46	354.00 ± 273.81
PND 21	289.30 ± 153.71	204.05 ± 86.07	210.65 ± 115.47	190.85 ± 112.70
PND 61	793.80 ± 240.48	807.00 ± 211.46	873.50 ± 246.17	965.60 ± 242.13
Females				
PND 13	171.55 ± 346.05	92.00 ± 215.34	64.40 ± 70.03	76.90 ± 122.61
PND 17	253.05 ± 233.18	327.05 ± 356.82	345.90 ± 224.90	266.70 ± 193.51
PND 21	213.35 ± 123.87	231.25 ± 142.71	273.15 ± 219.15	194.60 ± 112.69
PND 61	926.40 ± 218.70	986.50 ± 363.11	985.10 ± 418.05	1078.90 ± 336.24

[a] Data obtained from pages 281-296, MRID 46750501.
N = 20

TABLE 12e. Ambulatory activity sub-sessions - males [mean number of counts ± S.D.] [a]

Sub-session		Diet concentration (ppm)			
		0	50	100	125
PND 13	0-15	72.70 ± 112.53	34.75 ± 37.90	32.50 ± 39.48	44.05 ± 42.75
	16-30	22.75 ± 56.65	11.90 ± 29.38	38.40 ± 75.26	24.85 ± 84.23
	31-45	6.80 ± 16.11	3.70 ± 6.51	37.50 ± 76.69	30.75 ± 125.79
	46-60	16.05 ± 50.39	6.95 ± 12.50	30.15 ± 60.22	29.85 ± 80.74
PND 17	0-15	127.10 ± 82.04	180.85 ± 99.96	135.90 ± 118.55	162.25 ± 125.49
	16-30	43.40 ± 67.80	112.35 ± 118.04	71.90 ± 87.42	75.80 ± 80.15
	31-45	47.50 ± 75.59	93.90 ± 136.15	48.05 ± 80.13	49.20 ± 67.40
	46-60	43.70 ± 89.63	80.80 ± 122.91	43.85 ± 69.86	66.75 ± 87.89
PND 21	0-15	194.25 ± 66.96	169.95 ± 60.90	154.15 ± 63.70	157.85 ± 69.98
	16-30	44.30 ± 51.17	22.00 ± 31.94	29.55 ± 36.65	23.15 ± 37.00
	31-45	27.35 ± 42.68	10.65 ± 23.32	16.00 ± 45.80	4.85 ± 18.47
	46-60	23.40 ± 65.48	1.45 ± 4.94	10.95 ± 36.55	5.00 ± 17.80
PND 61	0-15	409.70 ± 107.30	440.30 ± 113.47	452.10 ± 101.63	469.95 ± 92.39
	16-30	171.30 ± 77.15	177.40 ± 67.89	180.90 ± 72.31	211.85 ± 69.41
	31-45	105.05 ± 62.79	113.20 ± 65.23	146.65 ± 72.52	154.50 ± 68.33
	46-60	107.75 ± 86.90	76.10 ± 60.30	93.85 ± 80.04	129.30 ± 106.26

[a] Data obtained from pages 281-296, MRID 46750501.
N = 20
* Significantly different from the control value, $p<0.05$.

TABLE 12f. Ambulatory activity sub-sessions – females [mean number of counts ± S.D.] [a]

Sub-session		Diet concentration (ppm)			
		0	50	100	125
PND 13	0-15	62.70 ± 110.97	35.10 ± 47.20	25.50 ± 26.84	43.80 ± 82.85
	16-30	61.40 ± 136.58	18.20 ± 41.35*	13.95 ± 22.31*	9.40 ± 25.50*
	31-45	33.40 ± 93.94	15.05 ± 61.0	16.40 ± 37.87	3.30 ± 5.39
	46-60	14.05 ± 42.07	23.65 ± 91.53	8.55 ± 12.83	20.40 ± 62.39
PND 17	0-15	123.55 ± 79.51	137.43 ± 128.17	182.85 ± 108.91	122.25 ± 70.74
	16-30	61.70 ± 73.63	57.67 ± 79.79	78.15 ± 83.41	34.10 ± 49.64
	31-45	42.90 ± 63.96	58.71 ± 98.95	42.60 ± 61.89	48.30 ± 66.46
	46-60	24.90 ± 66.40	73.24 ± 124.36	42.30 ± 70.81	62.05 ± 71.72
PND 21	0-15	154.10 ± 50.78	166.10 ± 64.58	183.70 ± 74.96	150.80 ± 64.53
	16-30	25.65 ± 32.11	32.65 ± 42.30	34.40 ± 61.92	25.00 ± 31.66
	31-45	24.85 ± 40.98	21.00 ± 36.80	33.50 ± 67.46	12.20 ± 24.51
	46-60	8.75 ± 25.09	11.50 ± 28.65	21.55 ± 38.38	6.60 ± 20.61
PND 61	0-15	455.50 ± 67.81	477.15 ± 98.02	477.90 ± 96.64	495.40 ± 105.87
	16-30	200.15 ± 89.46	244.00 ± 127.19	209.90 ± 120.67	269.20 ± 98.33
	31-45	142.50 ± 94.13	151.40 ± 128.25	170.30 ± 128.23	168.40 ± 96.93
	46-60	128.25 ± 88.98	113.95 ± 92.24	127.00 ± 143.20	145.90 ± 114.47

[a] Data obtained from pages 281-296, MRID 46750501.
N = 20
* Statistically different from control, $p<0.05$

b. **Auditory startle reflex habituation:** The overall amplitude and latency data are presented in Table 13a. Interval amplitude and latency data are included in Tables 13b (males) and 13c (females). On PND 20, mean overall latency to maximum response

(T_{MAX}) was statistically significantly increased in females at 100 and 125 ppm. However, this increase was not dose dependent. Mean T_{MAX} was statistically significantly and dose-dependently increased in females during the 1-10 minute interval on PND 20 at 100 and 125 ppm; however, since the increase was not observed at other intervals on PND 20, it was not considered toxicologically significant. In PND 60 males, the mean maximum response amplitude (V_{MAX}) was increased in a dose-dependent fashion; however, the increase was not considered treatment-related due to the high variability (CV>100%) around each mean and since a dose-dependent increase was not observed at any interval. Habituation was apparent, but not robust, in control and treated animals on PNDs 20 and 60. Habituation was less apparent in high-dose PND 60 males.

TABLE 13a. Mean (± SD) overall (Blocks 1-5) acoustic startle peak amplitude (mv), latency to peak (msec) and average response amplitude (mv) [a,b]

Diet conc. (ppm)	Parameter	Males		Females	
		PND 20	PND 60	PND 20	PND 60
0	V_{MAX}	106.95 ± 122.88	101.97 ± 214.96	129.17 ± 193.01	69.16 ± 152.20
	T_{MAX}	26.88 ± 14.46	31.52 ± 17.82	23.61± 10.07	31.47 ± 16.36
	V_{AVE}	19.76 ± 25.50	18.96 ± 43.19	23.63 ± 35.33	10.79 ± 27.03
50	V_{MAX}	112.58 ± 122.77	124.25 ± 219.95	147.61 ± 176.71	82.73 ± 171.31
	T_{MAX}	25.31 ± 9.33	31.14 ± 17.35	23.63 ± 7.89	32.01 ± 16.60
	V_{AVE}	22.31 ± 28.59	23.78 ± 43.54	28.79 ± 37.60	14.45 ± 33.50
100	V_{MAX}	91.06 ± 96.89	125.06 ± 286.22	106.47± 130.51	63.65 ± 121.34
	T_{MAX}	27.96 ± 9.75	29.91 ± 17.62	26.04 ± 12.06*	32.96 ± 20.23
	V_{AVE}	17.12 ± 21.85	22.63 ± 54.22	20.50 ± 27.88	9.80 ± 19.17
125	V_{MAX}	113.68 ± 132.91	140.22 ± 301.40	117.20 ± 135.71	78.59 ± 135.94
	T_{MAX}	26.63 ± 13.64	32.51 ± 16.28	25.02 ± 10.39*	33.33 ± 14.14
	V_{AVE}	21.50 ± 27.82	26.55 ± 56.93	21.89 ± 28.81	13.19 ± 26.22

[a] Data were obtained from pages 297-308, MRID 46750501.
[b] Overall SDs calculated by EPA reviewer as the square root of the sum of variances for blocks 1-5 (Tables 13b and c)
N= 20
* Significantly different from the control value, $p<0.05$.
V_{MAX} = maximum response amplitude
T_{MAX} = latency to V_{MAX}
V_{AVE} = average response amplitude.

TABLE 13b. Mean (±SD) interval acoustic startle peak amplitude (mv), latency to peak (msec) and average response amplitude (mv) in males[a]

Diet conc. (ppm)	Parameter	1-10	11-20	21-30	31-40	41-50
PND 20						
0	V_{MAX}	140.65 ± 64.15	110.15 ± 58.75	102.62 ± 57.17	93.23 ± 48.11	88.12 ± 44.18
	T_{MAX}	28.84 ± 8.43	27.80 ± 7.72	26.84 ± 5.21	25.98 ± 5.15	24.93 ± 4.97
	V_{AVE}	27.17 ± 12.32	20.33 ± 12.09	18.70 ± 11.53	16.68 ± 10.62	15.95 ± 10.33
50	V_{MAX}	130.86 ± 59.59	111.94 ± 54.35	109.01 ± 66.75	98.82 ± 58.80	112.29 ± 49.22
	T_{MAX}	27.01 ± 4.68	25.88 ± 4.87	24.37 ± 3.23	24.90 ± 4.53	24.39 ± 3.23
	V_{AVE}	26.07 ± 12.76	21.70 ± 12.76	20.52 ± 13.38	19.79 ± 13.20	23.48 ± 11.78
100	V_{MAX}	117.41 ± 55.93	90.11 ± 42.94	86.25 ± 37.27	80.45 ± 35.00	81.10 ± 42.45
	T_{MAX}	32.83 ± 4.25	29.30 ± 4.84	26.77 ± 4.11	26.44 ± 5.03	24.47 ± 3.37
	V_{AVE}	22.91 ± 12.59	16.82 ± 9.77	15.39 ± 7.78	14.82 ± 7.44	15.65 ± 10.38
125	V_{MAX}	141.70 ± 65.92	105.40 ± 61.68	100.21 ± 59.50	109.49 ± 58.22	111.64 ± 50.85
	T_{MAX}	30.16 ± 9.61	26.75 ± 4.52	27.03 ± 5.64	24.62 ± 4.13	24.60 ± 4.94
	V_{AVE}	27.46 ± 13.44	19.54 ± 12.96	18.11 ± 12.38	20.07 ± 11.97	22.35 ± 11.36
PND 60						
0	V_{MAX}	145.28 ± 122.05	96.11 ± 60.93	106.27 ± 125.64	83.12 ± 86.91	79.07 ± 65.29
	T_{MAX}	31.76 ± 8.35	31.28 ± 6.46	30.25 ± 9.00	33.17± 7.63	31.12 ± 8.18
	V_{AVE}	28.03 ± 26.73	18.14 ± 13.11	19.47 ± 23.53	14.37 ± 15.18	14.82 ± 13.96
50	V_{MAX}	170.30 ± 94.79	127.99 ± 88.07	120.93 ± 98.61	91.32 ± 83.27	110.70 ± 122.38
	T_{MAX}	27.87 ± 3.69	31.29 ± 5.92	32.15 ± 9.39	31.33 ± 9.55	33.05 ± 8.55
	V_{AVE}	34.61 ± 20.84	24.57 ± 18.28	22.96 ± 18.99	16.77 ± 15.37	20.00 ± 23.03
100	V_{MAX}	157.51 ± 110.75	122.40 ± 134.12	117.14 ± 134.08	119.84 ± 145.40	108.45 ± 112.01
	T_{MAX}	30.65 ± 6.54	30.97 ± 10.11	31.68 ± 10.93	29.36 ± 4.96	26.90 ± 4.64
	V_{AVE}	30.80 ± 22.33	21.51 ± 24.33	20.35 ± 24.80	21.46 ± 27.93	19.04 ± 21.31
125	V_{MAX}	177.94 ± 157.72	112.44 ± 81.94	140.18 ± 175.20	137.86 ± 134.55	132.69 ± 102.25
	T_{MAX}	32.42 ± 5.59	34.22 ± 9.18	31.93 ± 7.23	32.67 ± 7.86	31.32 ± 5.97
	V_{AVE}	36.90 ± 33.45	21.65 ± 16.57	25.56 ± 29.53	24.88 ± 25.21	23.77 ± 18.44

[a] Data were obtained from pages 297-308, MRID 46750501.
N= 20
V_{MAX} = maximum response amplitude
T_{MAX} = latency to V_{MAX}
V_{AVE} = average response amplitude.

TABLE 13c. Mean (±SD) interval acoustic startle peak amplitude (mv), latency to peak (msec) and average response amplitude (mv) in females[a]

Diet conc. (ppm)	Parameter	1-10	11-20	21-30	31-40	41-50
PND 20						
0	V_{MAX}	159.00 ± 95.35	141.64 ± 89.42	123.97 ± 83.88	119.61 ± 91.27	101.62 ± 69.29
	T_{MAX}	23.80 ± 3.77	23.36 ± 5.11	23.82 ± 4.48	22.54 ± 3.88	24.55 ± 5.08
	V_{AVE}	29.49 ± 17.30	25.81 ± 16.24	22.73 ± 16.23	21.53 ± 16.50	18.60 ± 12.24
50	V_{MAX}	181.84 ± 84.71	142.68 ± 74.83	134.57 ± 80.46	138.22 ± 80.07	140.73 ± 74.62
	T_{MAX}	24.84 ± 3.79	24.46 ± 4.60	23.97 ± 3.29	23.01 ± 3.36	21.87 ± 2.14
	V_{AVE}	34.95 ± 18.21	28.14 ± 15.82	25.51 ± 16.30	26.39 ± 16.39	28.95 ± 17.26
100	V_{MAX}	136.71 ± 69.19	109.84 ± 58.00	97.54 ± 59.61	90.84 ± 46.32	97.43 ± 56.42
	T_{MAX}	28.14 ± 5.96*	26.93 ± 7.24	26.65 ± 5.26	24.81 ± 4.51	23.67 ± 3.07
	V_{AVE}	26.46 ± 14.13	21.38 ± 12.61	18.74 ± 13.03	17.58 ± 10.31	18.36 ± 11.94
125	V_{MAX}	139.90 ± 54.75	125.92 ± 68.96	104.60 ± 58.74	100.90 ± 56.79	114.67 ± 63.16
	T_{MAX}	29.14 ± 4.95*	22.90 ± 3.73	25.95 ± 6.92	23.54 ± 2.95	23.59 ± 3.60
	V_{AVE}	26.30 ± 11.52	22.76 ± 14.59	19.32 ± 12.73	18.44 ± 11.97	22.64 ± 13.39
PND 60						
0	V_{MAX}	103.71 ± 80.30	69.61 ± 66.22	52.93 ± 40.59	63.99 ± 82.18	55.59 ± 62.70
	T_{MAX}	31.16 ± 3.02	32.08 ± 7.34	33.47 ± 8.55	30.59 ± 6.97	30.05 ± 9.12
	V_{AVE}	17.21 ± 15.42	10.45 ± 10.23	8.12 ± 7.27	10.03 ± 14.89	8.13 ± 10.66
50	V_{MAX}	119.03 ± 90.34	81.87 ± 50.47	79.43 ± 69.31	68.02 ± 89.72	65.30 ± 76.06
	T_{MAX}	30.67 ± 6.83	31.24 ± 5.98	31.21 ± 9.35	32.88 ± 7.30	34.06 ± 7.24
	V_{AVE}	22.21 ± 18.11	14.21 ± 9.79	13.62 ± 14.29	11.18 ± 16.81	11.04 ± 14.55
100	V_{MAX}	98.44 ± 65.34	60.98 ± 61.69	47.20 ± 36.77	62.43 ± 57.37	49.23 ± 44.77
	T_{MAX}	31.28 ± 6.23	32.60 ± 9.73	35.47 ± 11.55	33.02 ± 7.57	32.46 ± 9.23
	V_{AVE}	16.07 ± 11.32	9.46 ± 9.42	6.76 ± 5.62	9.30 ± 8.34	7.42 ± 7.02
125	V_{MAX}	123.59 ± 54.78	92.36 ± 101.61	53.67 ± 30.37	61.18 ± 37.17	62.16 ± 53.40
	T_{MAX}	30.42 ± 4.21	34.24 ± 5.83	33.92 ± 6.32	33.14 ± 7.22	34.96 ± 7.49
	V_{AVE}	22.16 ± 11.24	15.97 ± 20.27	8.59 ± 5.79	9.89 ± 6.65	9.36 ± 8.51

[a] Data were obtained from pages 297-308, MRID 46750501.
N= 20
* Significantly different from control value, $p<0.05$.
V_{MAX} = maximum response amplitude
T_{MAX} = latency to V_{MAX}
V_{AVE} = average response amplitude.

d. <u>Learning and memory testing</u>: No treatment-related changes were observed in swimming ability on the first day of testing (PND 25 or 62) or in the times to criterion (mean time to locate the submerged platform) during the learning and memory trials. The mean number of errors during the various phases of testing was unaffected by treatment. Data are presented in Tables 14 (PND 25) and 15 (PND 62).

TABLE 14. Water maze performance (mean ± S.D.) in offspring on PND 25 [a]

Session/parameter	Diet concentration (ppm)			
	0	50	100	125
Males				
Day 1 – St Channel escape time (secs)				
Trial 1	18.97 ± 13.83	16.91 ± 6.74	16.74 ± 7.50	15.66 ± 7.08
Trial 2	7.36 ± 3.16	8.19 ± 5.08	8.10 ± 2.87	7.47 ± 2.64
Trial 3	6.24 ± 1.98	6.31 ± 2.17	5.86 ± 1.80	5.59 ± 0.79
Trial 4	5.75 ± 1.93	6.05 ± 1.69	6.38 ± 2.56	5.34 ± 1.57
Day 2 – Trial 1 – Path A				
Mean Time (secs)	61.66 ± 28.27	72.31 ± 41.91	73.66 ± 51.68	58.46 ± 28.85
Mean No. Errors	13.90 ± 7.89	16.50 ± 9.78	17.10 ± 12.54	13.25 ± 9.12
Day 2 – Trial 2 – Path A				
Mean Time (secs)	50.47 ± 28.22	65.78 ± 43.77	47.56 ± 27.32	49.78 ± 40.26
Mean No. Errors	11.80 ± 9.43	13.60 ± 10.51	9.80 ± 7.22	11.75 ± 13.01
Day 3 – Trial 3 – Path A				
Mean Time (secs)	43.18 ± 42.02	33.40 ± 19.27	34.09 ± 16.61	44.44 ± 34.24
Mean No. Errors	9.90 ± 12.38	6.90 ± 6.18	7.60 ± 6.52	10.05 ± 9.80
Day 3 – Trial 4 – Path A				
Mean Time (secs)	42.05 ± 41.06	39.74 ± 27.30	34.62 ± 25.34	34.07 ± 20.13
Mean No. Errors	9.25 ± 13.28	8.45 ± 7.98	7.25 ± 7.21	7.05 ± 6.75
Day 4 – Trial 5 – Path B				
Mean Time (secs)	154.98 ± 41.73	129.79 ± 57.49	155.90 ± 42.61	146.15 ± 52.04
Mean No. Errors	34.25 ± 13.16	28.25 ± 13.96	33.50 ± 11.91	31.65 ± 12.75
Day 4 – Trial 6 – Path B				
Mean Time (secs)	107.34 ± 62.64	109.46 ± 69.59	122.14 ± 55.01	116.41 ± 65.63
Mean No. Errors	24.55 ± 15.88	24.90 ± 17.16	26.75 ± 11.68	26.30 ± 15.61
Day 5 – Trial 7 – Path B				
Mean Time (secs)	117.04 ± 57.25	81.72 ± 55.23	88.11 ± 61.14	99.95 ± 68.47
Mean No. Errors	26.30 ± 15.07	15.95 ± 11.51	17.45 ± 13.89	20.40 ± 16.46
Day 5 – Trial 8 – Path B				
Mean Time (secs)	63.10 ± 56.66	71.72 ± 51.83	76.64 ± 58.93	74.84 ± 60.92
Mean No. Errors	11.85 ± 12.44	12.60 ± 9.25	14.35 ± 12.86	13.25 ± 11.69
Day 6 – Trial 9 – Path B				
Mean Time (secs)	69.08 ± 61.14	60.92 ± 54.00	49.15 ± 39.64	55.56 ± 56.45
Mean No. Errors	12.35 ± 12.97	11.50 ± 11.56	9.25 ± 9.62	10.15 ± 12.59
Day 6 – Trial 10 – Path B				
Mean Time (secs)	50.48 ± 41.33	60.60 ± 55.62	58.83 ± 59.08	63.56 ± 64.22
Mean No. Errors	10.55 ± 11.00	9.85 ± 8.97	11.25 ± 12.92	10.85 ± 13.75
Day 7 – Trial 11 – Path A (Probe)				
Mean Time (secs)	68.52 ± 37.70	61.35 ± 40.15	63.08 ± 34.07	70.12 ± 47.18
Mean No. Errors	16.30 ± 8.68	14.70 ± 13.00	15.45 ± 10.39	14.75 ± 10.87
Day 7 – Trial 12 – Path A (Probe)				
Mean Time (secs)	56.63 ± 32.80	47.95 ± 23.44	61.37 ± 45.06	56.24 ± 37.60
Mean No. Errors	11.55 ± 7.74	10.65 ± 8.15	13.40 ± 12.79	12.30 ± 11.11
Females				
Day 1 – St Channel Escape Time (secs)				
Trial 1	14.61 ± 5.80	15.79 ± 5.51	23.50 ± 17.77	16.65 ± 8.39
Trial 2	7.12 ± 3.02	6.82 ± 2.17	6.72 ± 1.86	7.91 ± 2.99
Trial 3	5.65 ± 1.47	5.36 ± 1.21	5.58 ± 1.72	6.18 ± 1.91
Trial 4	5.24 ± 1.09	6.03 ± 2.10	5.67 ± 1.72	6.45 ± 2.31
Day 2 – Trial 1 – Path A				

TABLE 14. Water maze performance (mean ± S.D.) in offspring on PND 25 [a]				
Session/parameter	**Diet concentration (ppm)**			
	0	**50**	**100**	**125**
Mean Time (secs)	81.89 ± 45.25	53.88 ± 18.95	53.27 ± 45.84	64.49 ± 43.71
Mean No. Errors	18.60 ± 11.47	11.75 ± 5.71	11.70 ± 11.35	14.30 ±11.25
Day 2 – Trial 2 – Path A				
Mean Time (secs)	45.55 ± 40.50	47.22 ± 31.74	61.08 ± 42.93	53.28 ± 27.68
Mean No. Errors	7.50 ± 7.72	9.80 ± 8.10	13.80 ± 10.03	11.95 ± 8.54
Day 3 – Trial 3 – Path A				
Mean Time (secs)	50.57 ± 27.10	60.42 ± 53.70	57.14 ± 38.88	48.80 ± 40.54
Mean No. Errors	10.90 ± 7.20	14.40 ± 14.95	12.70 ± 10.30	10.45 ± 9.89
Day 3 – Trial 4 – Path A				
Mean Time (secs)	40.44 ± 28.91	52.24 ± 22.49	47.57 ± 30.10	39.15 ± 23.55
Mean No. Errors	7.75 ± 7.18	11.70 ± 7.95	10.05 ± 8.36	7.85 ± 7.10
Day 4 – Trial 5 – Path B				
Mean Time (secs)	146.51 ± 50.08	165.29 ± 34.56	153.01 ± 50.66	140.93 ± 58.83
Mean No. Errors	30.35 ± 14.55	37.75 ± 11.70	34.65 ± 14.78	30.10 ± 12.56
Day 4 – Trial 6 – Path B				
Mean Time (secs)	118.80 ± 56.33	113.91 ± 60.69	111.18 ± 63.33	116.83 ± 67.75
Mean No. Errors	25.65 ± 13.85	23.45 ± 13.88	23.25 ± 15.91	23.95 ± 16.51
Day 5 – Trial 7 – Path B				
Mean Time (secs)	116.23 ± 64.05	114.73 ± 61.96	117.85 ± 65.73	96.15 ± 65.66
Mean No. Errors	23.35 ± 14.83	22.50 ± 14.06	23.60 ± 14.70	19.45 ± 17.14
Day 5 – Trial 8 – Path B				
Mean Time (secs)	82.72 ± 65.84	89.59 ± 68.07	78.17 ± 61.25	71.21 ± 60.40
Mean No. Errors	15.00 ± 13.40	14.45 ± 12.37	12.60 ± 11.60	11.50 ± 12.02
Day 6 – Trial 9 – Path B				
Mean Time (secs)	76.18 ± 54.89	75.25 ± 58.68	95.90 ± 71.14	71.79 ± 70.67
Mean No. Errors	15.60 ± 13.45	15.25 ± 14.84	18.65 ± 16.19	12.35 ± 13.68
Day 6 – Trial 10 – Path B				
Mean Time (secs)	55.41 ± 43.79	73.92 ± 58.24	71.28 ± 66.09	50.80 ± 57.86
Mean No. Errors	9.00 ± 8.35	14.00 ± 12.15	12.65 ± 14.54	7.40 ± 11.62
Day 7 – Trial 11 – Path A (Probe)				
Mean Time (secs)	85.39 ± 44.0	76.71 ± 51.24	70.30 ± 35.37	85.76 ± 40.64
Mean No. Errors	20.55 ± 11.98	17.05 ± 12.12	15.35 ± 9.74	17.60 ± 10.02
Day 7 – Trial 12 – Path A (Probe)				
Mean Time (secs)	60.03 ± 43.89	63.50 ± 37.56	55.84 ± 29.89	54.86 ± 30.88
Mean No. Errors	11.65 ± 9.72	12.85 ± 7.49	10.75 ± 7.57	10.05 ± 8.15

[a] Data obtained from pages 309-322, MRID 46750501.
St = straight
N = 20

TABLE 15. Water maze performance (mean ± S.D.) in offspring on PND 62 [a]

Session/Parameter	Diet concentration (ppm)			
	0	50	100	125
Males				
Day 1 – St Channel Escape Time (secs)				
Trial 1	7.92 ± 4.47	8.95 ± 5.33	8.09 ± 3.37	7.40 ± 2.59
Trial 2	5.12 ± 0.86	5.84 ± 3.81	5.96 ± 4.38	6.44 ± 5.30
Trial 3	5.34 ± 1.63	6.11 ± 4.16	5.03 ± 1.84	5.74 ± 3.07
Trial 4	6.31 ± 4.41	6.40 ± 3.68	7.24 ± 6.00	5.76 ± 4.89
Day 2 – Trial 1 – Path A				
Mean Time (secs)	72.20 ± 51.97	65.14 ± 51.34	59.67 ± 48.04	76.01 ± 60.84
Mean No. Errors	15.45 ± 11.47	13.80 ± 10.34	12.65 ± 9.23	15.35 ± 12.77
Day 2 – Trial 2 – Path A				
Mean Time (secs)	39.74 ± 33.14	52.13 ± 44.67	50.46 ± 43.94	52.97 ± 38.28
Mean No. Errors	9.00 ± 7.23	12.70 ± 12.66	11.65 ± 10.76	11.95 ± 7.67
Day 3 – Trial 3 – Path A				
Mean Time (secs)	33.13 ± 30.19	42.99 ± 38.60	37.48 ± 26.89	31.66 ± 24.16
Mean No. Errors	7.40 ± 6.78	11.15 ± 11.89	9.65 ± 8.55	6.85 ± 6.79
Day 3 – Trial 4 – Path A				
Mean Time (secs)	28.10 ± 33.17	21.92 ± 18.47	29.70 ± 25.79	29.00 ± 28.31
Mean No. Errors	5.45 ± 9.19	5.20 ± 6.54	7.50 ± 7.86	6.80 ± 8.56
Day 4 – Trial 5 – Path B				
Mean Time (secs)	138.66± 55.57	138.46 ± 49.46	146.41 ± 55.36	146.63 ± 40.57
Mean No. Errors	31.15 ± 12.68	31.35 ± 10.39	29.60 ± 11.80	33.20 ± 10.02
Day 4 – Trial 6 – Path B				
Mean Time (secs)	111.59 ± 70.85	86.54 ± 68.56	119.82 ± 65.87	95.80 ± 69.39
Mean No. Errors	23.80 ± 15.94	17.65 ± 13.36	25.74 ± 13.39	18.40 ± 13.98
Day 5 – Trial 7 – Path B				
Mean Time (secs)	72.60 ± 46.70	97.48 ± 63.81	100.38 ± 67.20	69.66 ± 69.96
Mean No. Errors	13.75 ± 9.18	19.20 ± 11.77	18.75 ± 12.67	14.45 ± 16.12
Day 5 – Trial 8 – Path B				
Mean Time (secs)	46.12 ± 48.38	66.25 ± 59.33	54.23 ± 49.95	58.48 ± 59.48
Mean No. Errors	7.45 ± 8.33	11.60 ± 10.64	9.20 ± 7.94	9.90 ± 10.58
Day 6 – Trial 9 – Path B				
Mean Time (secs)	58.99 ± 56.79	40.09 ± 40.98	68.19 ± 54.08	40.49 ± 50.90
Mean No. Errors	11.75 ± 12.64	8.35 ± 7.39	13.15 ± 10.86	7.80 ± 10.15
Day 6 – Trial 10 – Path B				
Mean Time (secs)	35.68 ± 41.59	44.23 ± 52.93	32.32 ± 29.74	31.67 ± 51.29
Mean No. Errors	6.45 ± 7.46	8.50 ± 11.42	6.55 ± 7.39	4.75 ± 8.53
Day 7 – Trial 11 – Path A (Probe)				
Mean Time (secs)	85.20 ± 52.02	82.19 ± 56.48	97.55 ± 55.21	75.24 ± 45.19
Mean No. Errors	22.30 ± 13.33	22.10 ± 15.16	26.10 ± 15.32	19.10 ± 11.64
Day 7 – Trial 12 – Path A (Probe)				
Mean Time (secs)	66.88 ± 49.32	43.83 ± 30.22	60.10 ± 52.11	40.28 ± 28.34
Mean No. Errors	13.80 ± 9.64	11.30 ± 9.83	14.35 ± 11.66	9.95 ± 9.01
Females				
Day 1 – St Channel Escape Time (secs)				
Trial 1	9.64 ± 4.97	10.58 ± 5.17	15.23 ± 13.77	10.89 ± 2.96
Trial 2	5.93 ± 2.08	6.04 ± 2.23	7.25 ± 5.17	6.18 ± 2.41
Trial 3	5.41 ± 1.56	5.42 ± 1.25	6.00 ± 2.13	8.81 ± 9.03
Trial 4	5.09 ± 1.99	5.56 ± 2.09	5.04 ± 2.26	5.99 ± 2.93
Day 2 – Trial 1 – Path A				

TABLE 15. Water maze performance (mean ± S.D.) in offspring on PND 62 [a]				
Session/Parameter	**Diet concentration (ppm)**			
	0	**50**	**100**	**125**
Mean Time (secs)	69.08 ± 41.14	77.31 ± 42.48	79.66 ± 58.78	65.78 ± 44.79
Mean No. Errors	15.15 ± 9.62	15.70 ± 7.15	18.00 ± 14.44	15.45 ± 11.25
Day 2 – Trial 2 – Path A				
Mean Time (secs)	53.97 ± 34.16	50.67 ± 25.40	46.51 ± 28.98	52.91 ± 44.01
Mean No. Errors	12.20 ± 8.26	12.25 ± 7.95	11.05 ± 7.81	10.95 ± 9.85
Day 3 – Trial 3 – Path A				
Mean Time (secs)	31.94 ± 28.40	48.20 ± 43.68	30.33 ± 17.85	34.28 ± 15.75
Mean No. Errors	6.90 ± 8.86	10.75 ± 11.57	6.05 ± 5.16	8.50 ± 5.08
Day 3 – Trial 4 – Path A				
Mean Time (secs)	29.49 ± 19.60	20.71 ± 10.95	25.42 ± 19.88	27.83 ± 26.36
Mean No. Errors	5.55 ± 6.59	2.90 ± 3.40	5.20 ± 7.52	6.35 ± 9.20
Day 4 – Trial 5 – Path B				
Mean Time (secs)	142.42 ± 53.51	121.66 ± 52.21	125.59 ± 55.40	115.85 ± 54.76
Mean No. Errors	32.74 ± 12.33	27.75 ± 13.23	30.60 ± 14.42	31.30 ± 16.11
Day 4 – Trial 6 – Path B				
Mean Time (secs)	95.97 ± 69.24	93.04 ± 61.92	91.51 ± 60.48	60.88 ± 42.61
Mean No. Errors	19.50 ± 15.93	19.25 ± 12.90	19.30 ± 14.40	14.35 ± 10.34
Day 5 – Trial 7 – Path B				
Mean Time (secs)	87.15 ± 64.19	65.30 ± 59.93	78.95 ± 69.76	71.36 ± 65.04
Mean No. Errors	17.40 ± 12.87	12.75 ± 12.69	13.90 ± 13.19	13.35 ± 13.07
Day 5 – Trial 8 – Path B				
Mean Time (secs)	66.63 ± 60.80	42.58 ± 34.99	69.95 ± 62.10	50.79 ± 42.13
Mean No. Errors	11.30± 12.23	8.60 ± 8.85	12.55 ± 13.79	10.00 ± 7.95
Day 6 – Trial 9 – Path B				
Mean Time (secs)	36.99 ± 33.42	48.20 ± 43.10	63.65 ± 56.47	25.60 ± 15.16
Mean No. Errors	6.80 ± 8.06	10.35 ± 12.05	12.50 ± 12.49	4.70 ± 5.64
Day 6 – Trial 10 – Path B				
Mean Time (secs)	28.13 ± 21.63	31.78 ± 38.31	25.84 ± 16.40	23.91 ± 13.43
Mean No. Errors	4.70 ± 5.92	5.50 ± 12.50	4.00 ± 4.15	4.05 ± 4.07
Day 7 – Trial 11 – Path A (Probe)				
Mean Time (secs)	68.75 ± 51.14	54.32 ± 38.92	65.08 ± 47.64	56.43 ± 38.82
Mean No. Errors	17.65 ± 15.02	14.50 ± 14.24	16.85 ± 16.14	15.30 ± 11.37
Day 7 – Trial 12 – Path A (Probe)				
Mean Time (secs)	53.13 ± 42.32	28.18 ± 20.60	49.99 ± 34.27	34.98 ± 24.05
Mean No. Errors	13.05 ± 12.85	4.95 ± 5.46	13.05 ± 11.62	8.00 ± 8.14

[a] Data obtained from pages 323-336, MRID 46750501.
St = straight
N = 18-20

5. Postmortem results:

a. **Brain weight:** Mean brain weight data are presented Table 16. No treatment-related effects were noted at either PND 21 or 72.

TABLE 16. Mean (± SD) brain weight data [a]				
Parameter	Diet concentration (ppm)			
	0	50	100	125
Males				
Day 21				
Terminal body weight (g)	52 ± 5.7	51 ± 4.3	47 ± 4.4*	48 ± 6.1
Brain weight (g)	1.66 ± 0.06	1.64 ± 0.06	1.59 ± 0.08	1.62 ± 0.09
Brain-to-body weight ratio	3.23 ± 0.31	3.24 ± 0.31	3.44 ± 0.31	3.41 ± 0.43
Day 72				
Terminal body weight (g)	400 ± 29.0	404 ± 27.7	397 ± 24.4	406 ± 25.5
Brain weight (g)	2.13 ± 0.13	2.12 ± 0.11	2.11 ± 0.07	2.18 ± 0.09
Brain-to-body weight ratio	0.54 ± 0.04	0.53 ± 0.04	0.53 ± 0.03	0.54 ± 0.04
Females				
Day 21				
Terminal body weight (g)	49 ± 4.7	48 ± 7.1	44 ± 4.3*	49 ± 4.7
Brain weight (g)	1.55 ± 0.09	1.56 ± 0.09	1.54 ± 0.07	1.60 ± 0.05
Brain-to-body weight ratio	3.18 ± 0.27	3.31 ± 0.45	3.52 ± 0.33	3.28 ± 0.28
Day 72				
Terminal body weight (g)	253 ± 18.9	248 ± 17.5	239 ± 27.1	249 ± 14.2
Brain weight (g)	2.01 ± 0.09	2.02 ± 0.07	2.00 ± 0.13	1.98 ± 0.09
Brain-to-body weight ratio	0.80 ± 0.06	0.82 ± 0.06	0.84 ± 0.07	0.80 ± 0.04

[a] Data obtained from pages 339-342 and 355-358, MRID 46750501.
N = 15
* Significantly different from control value, $p<0.05$.

b. Neuropathology:

i. **Macroscopic examination:** No treatment-related lesions were observed.

ii. **Microscopic examination:** The incidences of microscopic findings for PNDs 21 and 72 are summarized in Table 17. Differences between control and high-dose groups were not analyzed statistically. At the PND 21 necropsy, 2/10 females at 125 ppm had minimal ectopic tissue (cluster of small dark cells) at the lateral margin of the caudoputamen (basal ganglia); none were observed in the control group. At the PND 72 necropsy, retinal dysplasia was observed in 2/10 males in the 125 ppm group compared to 0/10 in the control group. On this same day, minimal axonal degeneration of the lumbar dorsal root fibers was observed in 6/10 high-dose females (compared to 3/10 controls). Historical control data were not provided and are therefore requested for these microscopic observations.

TABLE 17. Incidence of select microscopic findings [a]				
Parameter	**Diet concentration (ppm)**			
	0	**50**	**100**	**125**
Males				
Day 72				
Lumbar dorsal root fibers Axonal degeneration, minimal	2/10	--	--	3/10
Lumbar ventral root fibers Axonal degeneration, minimal	0/10	--	--	1/10
Cervical dorsal root fibers Axonal degeneration, minimal	0/10	--	--	1/10
Sciatic nerve Axonal degeneration, minimal	8/10	--	--	6/10
Sural nerve Axonal degeneration, minimal	0/10	--	--	1/10
Tibial Nerve Axonal degeneration, minimal	4/10	--	--	3/10
Peroneal nerve Axonal degeneration, minimal	3/10	--	--	4/10
Eyes Retinal dysplasia, minimal	0/10	--	--	2/10
Hypothalamus Ectopic tissue, minimal	1/10	--	--	0/10
Females				
Day 21				
Basal Ganglia Ectopic tissue, minimal	0/10	--	--	2/10
Day 72				
Lumbar dorsal root fibers Axonal degeneration, minimal	3/10	--	--	6/10
Lumbar ventral root fibers Axonal degeneration, minimal	1/10	--	--	2/10
Sciatic nerve Axonal degeneration, minimal	5/10	--	--	4/10
Sural nerve Axonal degeneration, minimal	0/10	--	--	1/10
Eyes Retinal dysplasia, minimal	1/10	--	--	0/10

[a] Data obtained from pages 339-340, 349-352, 355-356 and 372-375, MRID 46750501.
-- = Not examined
N = 10

iii. **Brain Morphometry**: Morphometric data are presented in Table 18. A statistically significant decrease (3.5%) in the height of the hemisphere at Level 1 was observed in males at 125 ppm on PND 21. The change was not considered toxicologically significant given its small magnitude and the lack of other corroborating morphometric changes.

TABLE 18. Mean (cm ± SD) morphometric data [a]

Parameter	Diet concentration (ppm)			
	0	50	100	125
Males				
Day 21				
Brain Length (mm)	17.9 ± 0.36	17.8 ± 0.23	17.7 ± 0.28	17.8 ± 0.37
Brain Width (mm)	15.1 ± 0.26	15.1 ± 0.21	15.0 ± 0.34	15.1 ± 0.18
Level 1				
HT Hemisphere	0.659 ± 0.013	–	–	0.636 ± 0.014**
V Thickness Cortex	0.162 ± 0.007	–	–	0.157 ± 0.014
Level 3				
Radial thickness cortex	0.135 ± 0.006	–	–	0.134 ± 0.008
V HT BTW Hippocampal Pyr Neuron Layers	0.077 ± 0.008	–	–	0.075 ± 0.007
V HT Dentate Hilus	0.040 ± 0.005	–	–	0.041 ± 0.005
Length Ventral Limb Dentate Hilus	0.124 ± 0.018	–	–	0.123 ± 0.017
Level 5				
V Thickness of Pons	0.256 ± 0.034	–	–	0.247 ± 0.030
Base of Lobule 9	0.060 ± 0.007	–	–	0.058 ± 0.004
Day 72				
Brain Length (mm)	20.6 ± 0.41	20.5 ± 0.39	20.5 ± 0.24	20.5 ± 0.27
Brain Width (mm)	15.7 ± 0.35	15.6 ± 0.26	15.6 ± 0.28	15.7 ± 0.33
Level 1				
HT Hemisphere	0.642 ± 0.049	–	–	0.662 ± 0.035
V Thickness Cortex	0.161 ± 0.014	–	–	0.157 ± 0.008
Level 3				
Radial thickness cortex	0.163 ± 0.020	–	–	0.167 ± 0.009
V HT BTW Hippocampal Pyr Neuron Layers	0.087 ± 0.008	–	–	0.090 ± 0.004
V HT Dentate Hilus	0.048 ± 0.004	–	–	0.048 ± 0.004
Length Ventral Limb Dentate Hilus	0.145 ± 0.015	–	–	0.146 ± 0.014
Level 5				
V Thickness of Pons	0.279 ± 0.031	–	–	0.275 ± 0.028
Base of Lobule 9	0.062 ± 0.006	–	–	0.060 ± 0.008
Females				
Day 21				
Brain Length (mm)	17.6 ± 0.40	17.6 ± 0.33	17.5 ± 0.30	17.6 ± 0.34
Brain Width (mm)	14.8 ± 0.32	14.8 ± 0.36	14.8 ± 0.22	14.9 ± 0.17
Level 1				
HT Hemisphere	0.645 ± 0.030	–	–	0.646 ± 0.020
V Thickness Cortex	0.159 ± 0.010	–	–	0.161 ± 0.007
Level 3				
Radial thickness cortex	0.137 ± 0.006	–	–	0.139 ± 0.007
V HT BTW Hippocampal Pyr Neuron Layers	0.073 ± 0.006	–	–	0.075 ± 0.006
V HT Dentate Hilus	0.040 ± 0.003	–	–	0.041 ± 0.004
Length Ventral Limb Dentate Hilus	0.123 ± 0.011	–	–	0.133 ± 0.010
Level 5				
V Thickness of Pons	0.227 ± 0.022	–	–	0.218 ± 0.012
Base of Lobule 9	0.059 ± 0.007	–	–	0.059 ± 0.005
Day 72				
Brain Length (mm)	20.2 ± 0.32	20.1 ± 0.21	20.0 ± 0.44	19.9 ± 0.48
Brain Width (mm)	15.3 ± 0.28	15.2 ± 0.33	15.2 ± 0.40	15.4 ± 0.30
Level 1				
HT Hemisphere	0.647 ± 0.024	–	–	0.648 ± 0.014
V Thickness Cortex	0.156 ± 0.007	–	–	0.159 ± 0.008
Level 3				
Radial thickness cortex	0.160 ± 0.008	–	–	0.163 ± 0.008
V HT BTW Hippocampal Pyr Neuron Layers	0.086 ± 0.004	–	–	0.087 ± 0.004
V HT Dentate Hilus	0.047 ± 0.002	–	–	0.046 ± 0.002
Length Ventral Limb Dentate Hilus	0.134 ± 0.011	–	–	0.133 ± 0.006
Level 5	0.311 ± 0.039	–	–	0.288 ± 0.022
V Thickness of Pons	0.064 ± 0.006	–	-	0.063 ± 0.006

TABLE 18. Mean (cm ± SD) morphometric data [a]				
Parameter	Diet concentration (ppm)			
	0	50	100	125
Base of Lobule 9				

[a] Data obtained from pages 339-340, 349-352, 355-356 and 372-375, MRID 46750501.
HT = height; V = vertical; BTW = between; Pyr = pyramidal.
-- = Not examined
N = 10
** Statistically different from control, $p<0.01$

III. DISCUSSION and CONCLUSIONS:

A. INVESTIGATORS' CONCLUSIONS:

The study author concluded that neurobehavioral signs of toxicity occurred in the absence of signs of general systemic toxicity, e.g., decreased body weight. Specifically, treatment-related signs of maternal neurotoxicity at 100 and 125 ppm included increased grooming counts and tremors and/or clonic convulsions during the gestational and lactational FOB assessments, as well as tremors during the clinical observations of lactation. In the F_1 offspring, tremors and/or clonic convulsions were noted pre-weaning at 125 ppm, as were changes in auditory startle and motor activity at 100 and 125 ppm. The study author also concluded that treatment-related changes in offspring motor activity and auditory startle response were observed at 100 and 125 ppm, but without a clear relationship to dosing.

B. REVIEWER COMMENTS:

Administration of technical grade bifenthrin in the diet at concentrations of up to 125 ppm (9.0 and 20.7 mg/kg/day during gestation and lactation, respectively) produced no adverse effects on maternal body weight, body weight gain, and food consumption. Evidence of neurotoxicity in mid- and high-dose dams included tremors, increased grooming counts, and clonic convulsions. High-dose females also had an increased incidence of piloerection. Reproductive performance was unaffected by treatment.

The maternal LOAEL for bifenthrin in rats was 100 ppm (7.2 mg/kg/day during gestation and 16.2 mg/kg/day during lactation) based on clinical signs of neurotoxicity (tremors, clonic convulsions, and increased grooming counts). The maternal NOAEL is 50 ppm (3.6 mg/kg/day during gestation and 8.3 mg/kg/day during lactation).

Offspring survival was not affected by treatment. There was no treatment-related effect on offspring body weight and body weight gain or the mean day of attainment of sexual maturation. The cause of the lower mean body weight of the mid-dose pups during lactation is unknown, but the effect was not dose-related. Neurotoxicity was evident in offspring on PND 21 during the FOB, specifically as an increased incidence of tremors and clonic convulsions (limb tremors) in high-dose males and increased mean grooming counts in mid- and high-dose females. The increase in grooming counts was also noted in dams at the same dietary concentration that affected female offspring.

34

No treatment-related effects were observed during testing of motor activity, acoustic startle response, and learning and memory, as well as on brain weight, length, or width or at necropsy. At the PND 21 necropsy, 2/10 females at 125 ppm had minimal ectopic tissue (cluster of small dark cells) at the lateral margin of the caudoputamen (basal ganglia); none were observed in the control group. At the PND 72 necropsy, retinal dysplasia was observed in 2/10 males in the 125 ppm group compared to 0/10 in the control group. On this same day, minimal axonal degeneration of the lumbar dorsal root fibers was observed in 6/10 high-dose females (compared to 3/10 controls). Historical control data were not provided and are therefore requested for these microscopic observations. In brain morphometry, a statistically significant decrease (3.5%) in the height of the hemisphere at Level 1 was observed in males at 125 ppm on PND 21. The change was not considered toxicologically significant given its small magnitude and the lack of other corroborating morphometric changes.

The offspring LOAEL for bifenthrin in rats is 100 ppm (7.2 mg/kg/day during gestation and 16.2 mg/kg/day during lactation) based on clinical signs of neurotoxicity (increased grooming counts). The offspring NOAEL is 50 ppm (3.6 mg/kg/day during gestation and 8.3 mg/kg/day during lactation).

C. STUDY DEFICIENCIES:

- No details on the FOB procedures for maternal animals or offspring were provided.
- Historical control data were not provided and are therefore requested from relevant dietary studies for the neuropathological alterations reported in offspring above.
- If there was a deformation or irregularity of a region, morphometric analyses could not be performed on certain PND 21 or 72 animals. No explanation was provided as to the nature or cause of a deformation or irregularity.
- It was not stated why a more stringent statistical test was performed for non-monotonic dose responses (i.e., α=0.01) than for monotonic dose-response relationships (where α=0.05).

APPENDIX

STUDY TYPE: Range-finding study

PC CODE: 128825

TEST MATERIAL (PURITY): Bifenthrin (94.8% a.i.)

CITATION: Nemec, M. (2006) A dietary feasibility and range-finding study of bifenthrin technical in rats. WIL Research Laboratories, LLC, Ashland, OH. Study number WIL-105019, January 13, 2006. MRID 46750502. Unpublished .

EXECUTIVE SUMMARY: In a range-finding study (MRID 46750502), bifenthrin (94.8% a.i., Lot PL02-0477) was administered in the diet to 10 female Crl:CD®(SD)IGS BR rats per group at concentrations of 0, 50, 65, 80, 100 and 125 ppm (0, 3.6, 4.6, 6.0, 7.4 and 9.3 mg/kg/day, respectively, during gestation; 0, 9.2, 11.7, 14.3, 17.2 and 22.5 mg/kg/day, respectively, during lactation) from gestation day (GD) 6 through lactation day (LD) 22. Clinical observations were made and body weight and food consumption were measured in maternal animals. Females were allowed to deliver and rear their offspring until postnatal day (PND) 22. On postnatal day (PND) 4, litters were culled to yield four males and four females (as closely as possible). Pups were observed for morbidity and moribundity; other clinical observations were also recorded. Body weight was measured on PND 1, 4, 7, 11, 14, 17 and 22. Locomotor activity was assessed on one pup/sex/litter on PND 22.

On LDs 5, 11 and 17, milk samples were collected from all dams (following s.c. injection of oxytocin) and analyzed for the presence of bifenthrin. Blood was collected from five dams/group on LDs 4 and 22, and brain samples were collected on LD 22 from these same animals to analyze for the test material. The culled pups from these five dams were used for blood and brain collection on PND 4; blood and brain samples were pooled by litter. On PND 22, blood and brains were collected and necropsy was performed on one pup/sex/litter from the five selected litters; the remaining pups and all dams were discarded without examination. Brain weights in dams and offspring were recorded but unreported.

One female in the 50 ppm group delivered on GD 20 and was euthanized along with her pups. All other dams survived to scheduled termination. Slight to moderate whole-body tremors were observed in 8/10 dams (beginning as early as LD 5 and/or extending until LD 22) at 125 ppm. Body weight, food consumption, pregnancy rates, and gestation length were unaffected by treatment. No treatment-related clinical effects, macroscopic changes, or differences in motor activity were observed in the offspring.

Bifenthrin was detected in maternal milk and plasma and in offspring plasma on all sampling days. The highest mean concentration detected in milk occurred on LD 11 for all dietary levels (range of means: 6.01-10.4 ppm). Residue levels increased with increasing dietary concentration in a somewhat linear fashion. Mean maternal plasma levels were almost identical on LDs 4 and 22 and showed a more robust increase with increasing dose than did milk concentrations. Offspring plasma levels were similar to maternal levels, as were the dose-response and timecourse profiles. Mean concentrations of bifenthrin in milk and plasma samples are included

in Tables A-1 to A-3. Bifenthrin residues were not detected in milk or blood collected from control dams. Residues were found at the limit of detection (0.01 ppm) in the plasma of 2/5 control pups.

TABLE A-1: Mean levels of Bifenthrin (ppm) ± SD in maternal milk (range)					
Lactation Day Sample	**Diet concentration (N=9-10)**				
	50 ppm	**65 ppm**	**80 ppm**	**100 ppm**	**125 ppm**
Day 5	3.38 ± 0.73 (2.36 to 5.09)	5.05 ± 2.30 (1.18 to 8.56)	3.33 ± 1.58 (1.29 to 6.44)	5.11 ± 3.59 (1.08 to 10.1)	8.20 ± 4.06 (4.23 to 15.3)
Day 11	6.01 ± 1.60 (3.88 to 9.25)	7.94 ± 3.42 (4.00 to 14.9)	6.82 ± 3.27 (1.78 to 12.1)	10.1 ± 4.21 (5.30 to 18.7)	10.4 ± 5.45 (3.29 to 20.8)
Day 17	3.25 ± 1.62 (1.74 to 6.99)	4.45 ± 2.37 (2.06 to 9.13)	4.01 ± 1.78 (1.75 to 6.40)	3.60 ± 2.44 (0.90 to 8.36)	9.57 ± 2.67 (5.65 to 13.1)

Data taken from p. 15, MRID 46750502.

TABLE A-2: Mean levels of Bifenthrin (ppm) ± SD in maternal plasma (range)					
Lactation Day Sample	**Diet concentration (N=5)**				
	50 ppm	**65 ppm**	**80 ppm**	**100 ppm**	**125 ppm**
Day 4	0.13 ± 0.03 (0.08 to 0.16)	0.18 ± 0.07 (0.12 to 0.29)	0.17 ± 0.06 (0.08 to 0.22)	0.24 ± 0.06 (0.16 to 0.32)	0.33 ± 0.06 (0.20 to 0.49)
Day 22	0.13 ± 0.01 (0.12 to 0.15)	0.22 ± 0.02 (0.20 to 0.25)	0.20 ± 0.05 (0.14 to 0.26)	0.23 ± 0.03 (0.19 to 0.25)	0.30 ± 0.04 (0.23 to 0.34)

Data taken from p. 15, MRID 46750502.

TABLE A-3: Mean levels of Bifenthrin (ppm) ± SD in pup plasma (range)					
Lactation Day Sample	**Diet concentration**				
	50 ppm	**65 ppm**	**80 ppm**	**100 ppm**	**125 ppm**
Day 4 (litter means; N=5)	0.11 ± 0.02 (0.09 to 0.15)	0.12 ± 0.03 (0.08 to 0.15)	0.15 ± 0.02 (0.13 to 0.19)	0.19 ± 0.02 (0.17 to 0.21)	0.24 ± 0.06 (0.19 to 0.33)
Day 22 (per pup; one pup/sex; N=10)	0.11 ± 0.06 (0.05 to 0.22)	0.09 ± 0.06 (0.02 to 0.21)	0.12 ± 0.07 (0.04 to 0.25)	0.18 ± 0.05 (0.12 to 0.25)	0.13 ± 0.11 (0.05 to 0.41)

Data taken from p. 15, MRID 46750502.

13544

R133230

Chemical:	**Bifenthrin**
	PC Code: 128825
HED File Code:	**13000 Tox Reviews**
Memo Date:	**8/17/2006**
File ID:	***TX0054120***
Accession #:	**412-07-0024**

HED Records Reference Center
11/9/2006